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AP2 domain structure and protein motif features in Azerbaijan local durum and bread wheat genotypes

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DREB proteins belonging to the superfamily of AP2/ERF plant transcription factors play an important role in the signaling network that modulates many processes, such as stress responses and plant development. In the present study, we have isolated and molecularly characterized partial DREB1 gene from Azerbaijan's local durum and bread wheat genotypes, which are distinguished by tolerance to abiotic stress factors. Analysis of amino acid sequences encoded by the putative DREB genes revealed a strongly conserved AP2/ERF domain with two conserved functional amino acids (14th valine and 19th glutamic acid) which play crucial roles in the recognition of the DNA binding site. Nuclear localization signal and conserved Ser/Thr-rich region were observed in the corresponding amino acid sequences. One α-helix and two β-sheets were detected in the secondary structure of the AP2 domain. In the protein sequence with the AP2 domain, 3 and 11 amino acid substitutions were detected in bread wheat and durum wheat, respectively. The identified sequences of the DREB1 gene from durum and bread wheat are available in the GenBank database (Accession number MZ935737.1, MZ935738.1). Characterization of the DREB genes from the stress-tolerant wheat genotypes is important for further understanding the role of this gene in the plant stress-tolerance mechanisms. Moreover, the identified single nucleotide variations associated with stress tolerance can be used in genome editing for improving crops under stress conditions.

***Keywords**: DREB,* AP2/ERF*, NLS, Ser/Thr-rich region, durum wheat, bread wheat*

INTRODUCTION

According to large-scale transcriptome analyses, protective proteins and regulatory proteins are involved in molecular stress responses (Shahzad et al., 2021). Among them, transcription factors (TFs) were shown to play a crucial role in regulating plant growth and response to abiotic and biotic stresses. TFs are multi-functional proteins that may simultaneously control numerous pathways during stresses in plants-this makes them powerful tools for the manipulation of regulatory and stress-responsive pathways. Defining the structure-function relationships of numerous plant TFs involved in drought and associated stresses allowed the development of practical strategies for engineering plants with enhanced stress tolerance (Hrmova and Hussain, 2021). Recent studies have determined several main superfamilies of transcription factors involved in the stress-response reactions including myeloblastosis (MYB) oncogene, APETALA2/ethylene response factor (AP2/ERF), basic leucine zipper (bZIP), Cys2(C2)His2(H2)-type zinc fingers (ZFs), and transcription factors with a protein domain consisting of the conserved WRKYGQK (WRKY) motif (Lindemosa et al., 2013; Gao et al., 2018).

The AP2/ERF superfamily is characterized by the presence of an AP2/ERF DNA-binding domain of 60–70 amino acids, and it is composed

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of the ERF, AP2, RAV related to ABI3 (abscisic acid incentive 3) and VP1 (viviparous 1) families. ERF and AP2 family proteins consist of one (EREBP) and two (AP2 family) AP2/ERF domains, respectively, whereas RAV family proteins are composed of AP2/ERF domain and DNA binding B3 domain originally named due to its position in the third basic domain of the maize gene Viviparous1, VP1 (McCarty et al., 1991; Zhao et al., 2017; Gao et al., 2018). Results of the genome-wide studies indicate that the AP2/ERF transcription factors were highly conserved during plant evolution (Feng et al., 2020).

DREB, the Dehydration Responsive Element (DRE)-binding proteins family is one of the largest families of TFs that play a significant role in signaling networks modulating many plant processes (Agarwal et al., 2006; Lata and Prasad, 2011; Sarkar et al., 2019). The DREB proteins activate many abiotic stress-responsive genes and maintain water balance in plant systems thus imparting abiotic stress tolerance. The DREB TFs belonging to the AP2/ERF family of transcription factors were divided into two categories, DREB1 and DREB2. DREB1 is affected by low temperatures and DREB2 is induced by high salt and drought stresses (Zhuang et al., 2011; Filiz and Tombuloglu, 2014).

DREB1/CBF and DREB2 genes share a sequence similarity at the AP2 domain and bind to the 9 base pair sequence - C-repeat/DRE motif (TACCGACAT) in the promoter region of DREBs. The DRE element was first identified in the rd29A promoter, which contains a DRE core sequence (ACCGAC) (Yamaguchi-Shinozaki and Shinozaki, 1994; Li et al., 2018). All DREB genes have three conservative regions, such as the EREBP/AP2 DNA binding domain, the N-terminal nuclear localization signal, and the Ser/Thr-rich region. The two amino acids in the ERF/AP2 domain, valine (position 14) and glutamic acid (position 19) were found to play an important role in the DNA-binding specificity (Sakuma et al., 2002; Jan et al., 2017). It was an interesting point to find a DRE/CRT motif in a DREB promoter gene since it binds to the same motif in the promoter region of downstream stress-inducible genes. So, it can be predicted that the expression of the DREB gene is regulated by some other transcription factors such as DREB which interact with this unique motif. It was found that the DREB gene is also expressed under non-stress conditions, which may be related to the other functions of stress-inducible genes (Latini et al., 2008). Hence, to explore mechanisms of plants' tolerance to certain stress factors, cloning and sequencing of the DREB genes from different plant varieties with further comparative structural and functional studies seem to be one of the efficient ways to explore the role of DREBs.

The main goal of the study was the isolation and molecular characterization of the DREB gene from the Azerbaijan local wheat cultivars Barakatli 95 and Azamatli 95 which are distinguished by high productivity, quality, and tolerance to extreme factors of the environment.

MATERIALS AND METHODS

Plant materials. Barakatli 95 genotype of durum wheat (*Triticum durum* Desf., AABB, 2n = 4x = 28) and Azamatli 95 genotype of bread wheat (*Triticum aestivum* L., AABBDD, 2n = 6x = 42) were used. Barakatli 95 has been obtained at the Research Institute of Crop Husbandry (Baku, Azerbaijan) by individual selection from intraspecific hybridization of the local folk selection varieties Gyrmyzy Bugda and Garagylchyg due to their high productivity, quality and tolerance to abiotic stress factors. Azamatli 95 has been obtained by individual selection from bread wheat genotypes from the 16th elite variety testing seed plot (16 ESWYT-12) introduced from CIMMYT and adapted to local conditions (Aliyev et al., 2013).

DNA extraction and quantification. The DNA extraction was performed using the modified CTAB method (Murray and Thompson, 1980). The quantity of DNA was evaluated based on the optical density (OD) at λ=260 using the Epoch™ Microplate Spectrophotometer (BioTek, USA). The purity of the genomic DNA was determined by the ratio of absorptions at A260/A280. The quality of the DNA was checked on a 0.8% agarose gel stained with 10 mg/mL of ethidium bromide in 1×TBE (Tris base, Boric acid, EDTA) buffer. The gel was documented using the "Gel Documentation System UVITEK" (UK).

Table 1. Nucleotide sequence of the gene-specific primers

Primer description	Nucleotide sequence (5'- 3')	Product size, bp	Ann. Temp., ℃
PsDREB-F	TATGGATTGCCTTGATGAACA	500	53.3
PsDREB-R	GACTCCGATTCATCCTTCCC		

DNA amplification with gene-specific primers. Gene-specific primer pairs were used for isolation of the DREB gene (Pandey et al., 2014) (Table 1). DNA amplification was performed in a 25 μL reaction mixture volume, containing 10 x buffer, 20 ng of the genomic DNA, 0.2 μM primer, 200 μM of each of the following: dATP, dCTP, dGTP and dTTP, 2.5 mM $MgCl_2$, and 0.2 units of Taq-polymerase in the incubation buffer.

PCR was performed in the "Applied Biosystems 2720 Thermal Cycler" under the following conditions: 1 cycle - 5 min at 94ºC; 35 amplification cycles - 1 min at 94ºC, 1 min at 53.3ºC, 1 min at 72°C; the final elongation was performed at 72°C for 10 min, then kept at 4°C. The amplified products were electrophoresed on a 1.5% agarose gel, stained with ethidium bromide (EtBr), and visualized under ultraviolet light using "Gel Documentation System UVITEK" (UK).

Purification of PCR product and DNA sequencing. The preparative PCR amplification was carried out to amplify the DNA region of interest prior to Sanger sequencing. The amplification products were excised from the agarose gel and purified using ISOLATE II PCR & Gel Kit (BIOLINE) according to the manufacturer's instructions. The purified samples were then sequenced on an ABI 3130xl DNA analyzer (Applied Biosystems, USA).

Data analyses. To explore the protein-coding capacity of the sequenced DNA fragments and compare them with known genes/proteins, the FGENESH (*http://www.softberry.com/berry.phtml?topic=fgenesh&group=programs& subgroup=gfind*) (Solovyev et al., 2006) and BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Altschul et al., 1997) tools were used. To identify the conserved protein domains, the INTERPROSCAN *(http://www.ebi.ac.uk/Tools/pfa/iprscan/)* (Jones et al., 2014) and SMART *(http://smart.embl-heidelberg.de/*) (Letunic et al., 2015) programs were applied. A multiple amino acid sequence alignment was constructed using MAFFT v7.271 *(https://mafft.cbrc.jp/alignment/server/*) (Katoh and Standley, 2014). The protein secondary structure was predicted by PSIPRED method *(http://bioinf.cs.ucl.ac.uk/psipred/)*

RESULTS AND DISCUSSION

Clarification of gene functions and availability of wheat genome sequence information opens up new opportunities for improving crops under stress conditions (Rathan et al., 2021). In our previous studies, using the genome-specific functional markers in Azerbaijan wheat genotypes, a gene encoding DREB1 transcription factor was detected in the A, B, and D genomes (Huseynova et al., 2013). Wheat genotypes of Azerbaijan and German origin with contrasting drought tolerance were used to determine the expression level of the DREB1 transcription factor. In general, the transcript levels of all genotypes exposed to drought stress were found to increase significantly. Further, under drought stress, the expression level of DREB1 in the tolerant genotypes, durum wheat Barakatli 95 and bread wheat Azamatli 95, was increased more than in drought-sensitive ones (Rustamova et al., 2020). In the current study, we isolated a part of the DREB1 gene covering the AP2 domain from these genotypes. Fig.1 shows the electrophoretic profiles of PCR products obtained using gene-specific primer pair PsDREB-F/R. In addition to the expected 500 bp fragments in both genotypes, 300 bp fragments were also amplified. Amplification products were separated in 1.5% (w/v) agarose gel, purified using purification kit ISOLATE II PCR & Gel Kit (BIOLINE) and sequenced.

Further, the nucleotide sequences of the 500 bp and 300 bp DNA fragments were compared

with known DREB genes by the BLAST tool. It was found that the 500 bp fragment is highly similar to the DREB gene of other wheat genotypes from the GenBank (with 95% and 97% similarity levels for Barakatli 95 and Azamatli 95, respectively).

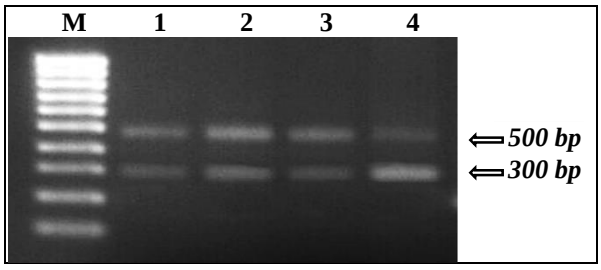

Fig. 1. Gel electrophoresis of PCR products of DREB1 gene in Barakatli 95(1-2) and Azamatli 95 (3-4). Arrows indicate the 300 bp and 500 bp fragments. M-100 bp DNA ladder.

A comparison with the Chinese Spring genome (the Ensembl assembly IWGSC) revealed four hits of significant similarity and three of them belong to the TraesCS3A02G099200, TraesCS3B02G115400, and TraesCS3D02G099500 genes located in the A, B, and D genomes, respectively. The TraesCS3A02G099200 gene has 1 transcript and encodes the DREB protein W73 (UniProtKB/TrEMBL; Q4U0C8) of 278 aa. Two alternative splice variants of the TraesCS3B02G115400 gene (B genome) encode proteins of 1008 aa and 1401 aa. The UniProtKB contains a single protein (Q3LR66) corresponding to this gene. The TraesCS3D02G099500 gene (D genome, chromosome 3) has 2 splice variants and encodes proteins of 1311 aa and 1225 aa. For this gene, the UniProtKB contains 2 proteins (G0YWB9; G0YWC2).

Comparison of putative DREB1 gene fragments from Barakatli 95 and Azamatli 95 genotypes with the reference DREB1 gene (DQ195068; https:// .ncbi.nlm.nih.gov/nuccore/DQ195068.1) by the MAFT tool revealed 88.42% similarity between them (Fig. 2).

Further, a search for possible genes/open reading frames (ORF) in the 500 bp DNA sequence of the durum wheat genotype Barakatli 95 by the FGENESH program predicted a gene fragment with one exon of 396 bp length (positions 16-412 positions). This fragment might encode a polypeptide of 132 amino acids (aa) in length. In the 500 bp sequence of bread wheat Azamatli 95, one exon of 381 bp length (positions 12-393) was predicted. This exon might encode a polypeptide of 127 aa length (Fig.3).

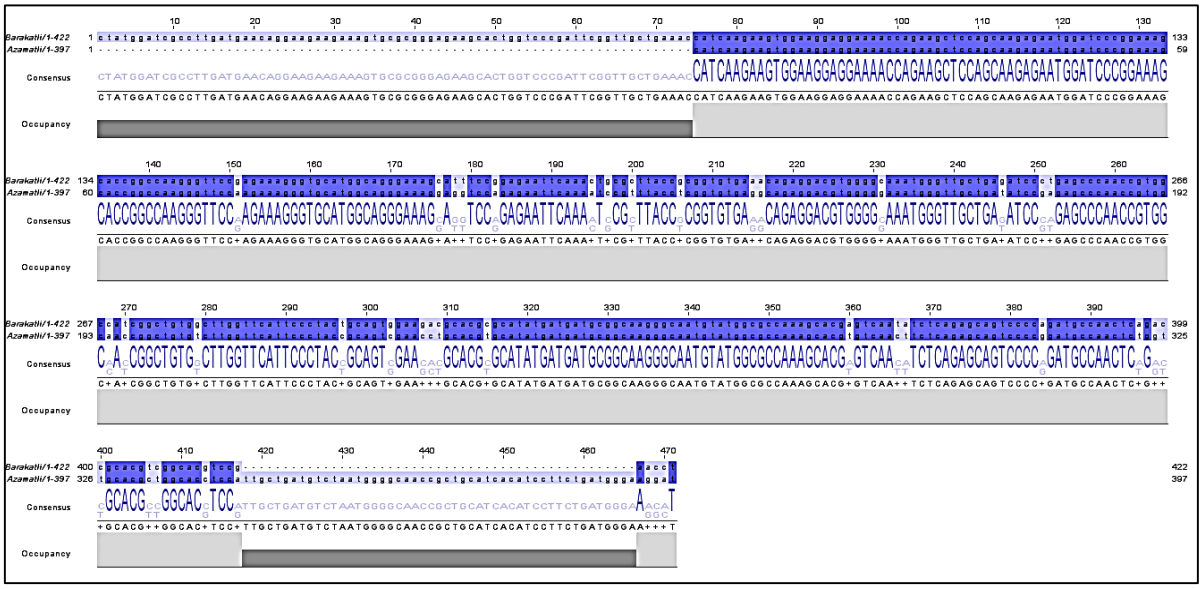

Fig. 2. Sequence alignment of the partial DREB1 gene isolated from Barakatli 95 and Azamatli 95 *using* MAFFT tool. Conservations of nucleotides are distinguished by various shades of blue color.

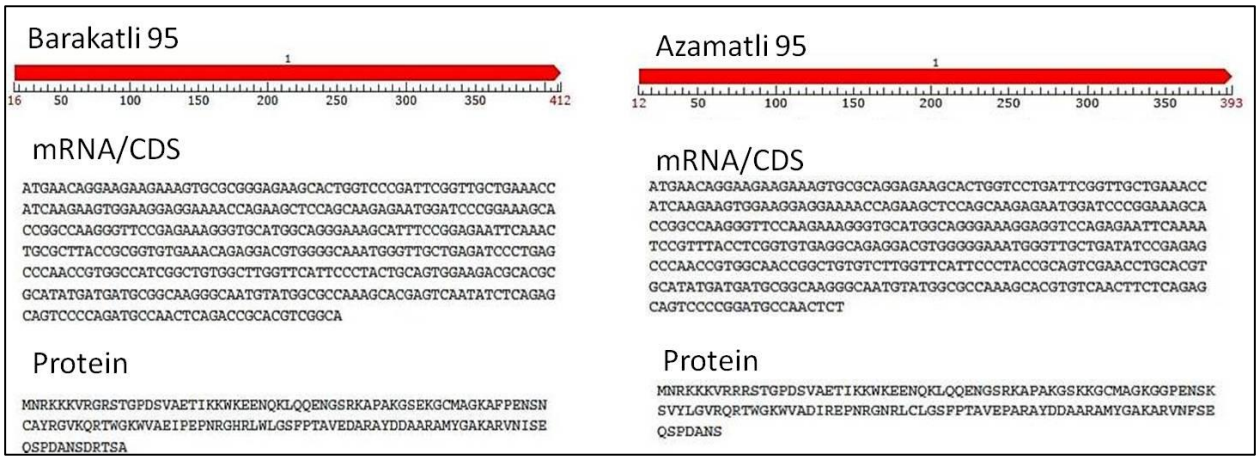

Fig. 3. Putative gene fragment (exon), CDS and protein in the 500 bp DNA sequence from Baraktli and Azamatli wheat genotypes predicted by the FGENESH.

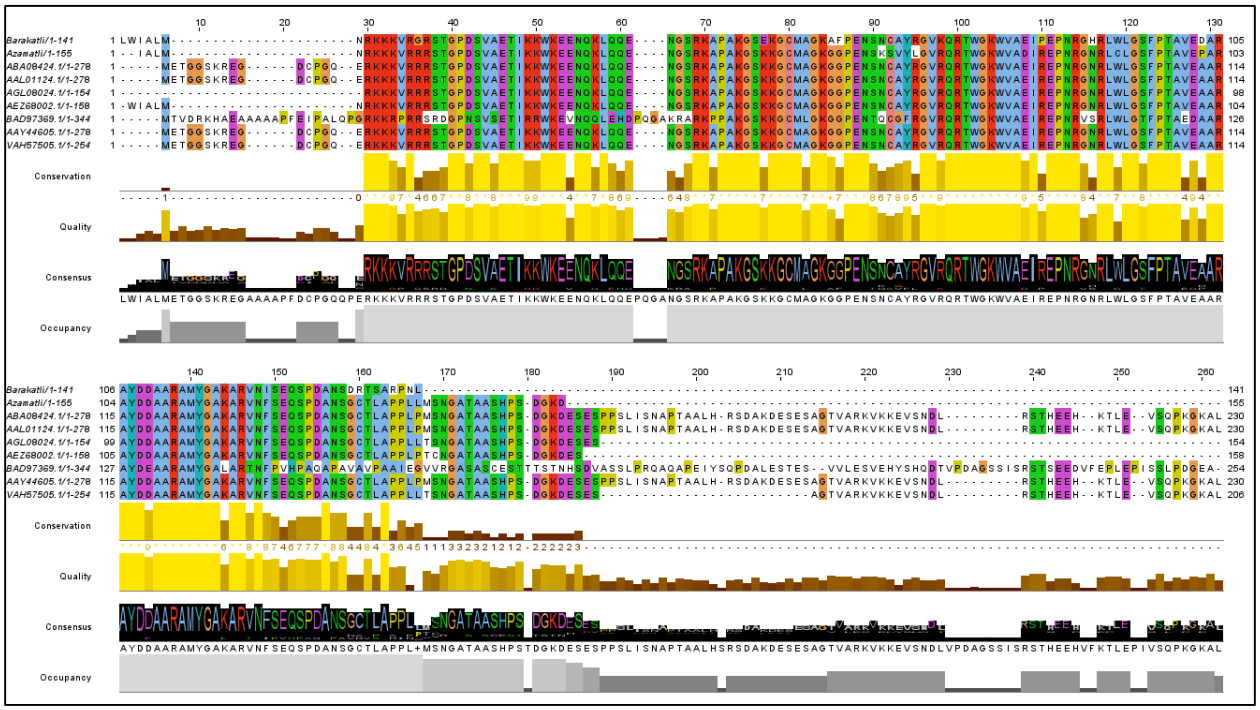

Fig. 4. Multiple alignment of DREB protein sequences by MAFFT tool.

To identify conserved regions, deduced amino-acid sequences of partial DREB genes and some AP2-containing proteins deposited in the NCBI GenBank, a multiple alignment of these sequences by MAFFT software was performed. Figure 4 describes the alignment of dehydration-responsive element binding proteins of *Triticum aestivum* L. (Azamatli 95, Accession Number AAL01124.1, ABA08424.1, AEZ68002.1, BAD97369.1), *Triticum durum* (Barakatli 95), *Triticum dicoccoides* (Accession Number AGL08024.1) and *Triticum turgidum subsp. Durum* (Accession number VAH57505.1) samples. As seen in the figure, single nucleotide variations (SNVs) available in the Barakatli 95 genotype result in amino acid substitutions in 11 points (positions 36, 77, 85, 86, 110, 116, 129, 159, 160, 164, and 166). In Azamatli 95 genotype amino acids were replaced in only 3 positions (91, 95, and 129).

Analysis of amino acid sequences encoded by the putative DREB1 genes from Barakatli 95 and Azamatli 95 by INTERPROSCAN and SMART computer programs revealed the AP2 domain in the 500 bp fragment with the two conserved functional amino acids (valine (V) and glutamic acid (E)) at the 14th and 19th residues which play crucial roles in recognition of the DNA-binding sequence (Fig. 5). However, some studies demonstrated that E19 might not be as necessary as V14 for this case (Sakuma et al., 2002; Rana et al., 2013). DREB proteins demonstrated a high level of identity, especially in the conserved regions. KKK and KKWK in the N-terminal region function as a nuclear localization signal (NLS). An entry of the nucleus-targeted transcription factors into the nucleus is regulated by the NLS (Akhtar et al., 2012; Pandey et al., 2014). A region of 56 amino acid residues, underlined with a solid line, is strongly conserved among DREB proteins. This region is referred to as the AP2/ERF DNA-binding domain. Two highly conserved functional amino acids at the 14th and 19th positions were also observed in the AP2 domain. These amino acids distinguish the DREB (valine and glutamic acid) from the ERF (alanine and aspartic acid) (Agarwal et al., 2007). Besides, tryptophan was found in the AP2 domain, followed by serine and threonine amino acids in polypeptides corresponding to the sequenced partial DREB gene. The results of our study are in line with data obtained by previous studies. The role of tryptophan rings in the recognition of GCC-box and determination of the geometry GCC-box binding domain was reported by Mondini et al. (2014).

```
Barakatli       LWIALM----------------------NRKKKVRGRSTGPDSVAETIKKWKEENQKLQQ
Azamatli        --IALM----------------------NRKKKVRRRSTGPDSVAETIKKWKEENQKLQQ
ABA08424.1      -----METGGSKREG------DCPGQ--ERKKKVRRRSTGPDSVAETIKKWKEENQKLQQ
AAL01124.1      -----METGGSKREG------DCPGQ--ERKKKVRRRSTGPDSVAETIKKWKEENQKLQQ
AGL08024.1      ----------------------------RKKKVRRRSTGPDSVAETIKKWKEENQKLQQ
AEZ68002.1      -WIALM----------------------NRKKKVRRRSTGPDSVAETIKKWKEENQKLQQ
BAD97369.1      -----MTVDRKHAEAAAAAPFEIPALQPGRKKRPRRSRDGPNSVSETIRRWKEVNQQLEH
AAY44605.1      -----METGGSKREG------DCPGQ--ERKKKVRRRSTGPDSVAETIKKWKEENQKLQQ
VAH57505.1      -----METGGSKREG------DCPGQ--ERKKKVRRRSTGPDSVAETIKKWKEENQKLQQ
                                        ***: *    **:**:***::*** **:*::

Barakatli       E----NGSRKAPAKGSEKGCMAGKAFPENSNCAYRGVKQRTWGKWVAEIREPNRGHRLWL
Azamatli        E----NGSRKAPAKGSKKGCMAGKGGPENSKSVYLGVRQRTWGKWVADIREPNRGNRLCL
ABA08424.1      E----NGSRKAPAKGSKKGCMAGKGGPENSNCAYRGVRQRTWGKWVAEIREPNRGNRLWL
AAL01124.1      E----NGSRKAPAKGSKKGCMAGKGGPENSNCAYRGVRQRTWGKWVAEIREPNRGNRLWL
AGL08024.1      E----NGSRKAPAKGSKKGCMAGKGGPENSNCAYRGVRQRTWGKWVAEIREPNRGNRLWL
AEZ68002.1      E----NGSRKAPAKGSKKGCMAGKGGPENSNCAYRGVRQRTWGKWVAEIREPNRGNRLWL
BAD97369.1      DPQGAKRARKPPAKGSKKGCMLGKGGPENTQCGFRGVRQRTWGKWVAEIREPNRVSRLWL
AAY44605.1      E----NGSRKAPAKGSKKGCMAGKGGPENSNCAYRGVRQRTWGKWVAEIREPNRGNRLWL
VAH57505.1      E----NGSRKAPAKGSKKGCMAGKGGPENSNCAYRGVRQRTWGKWVAEIREPNRGNRLWL
                :    : :**.*****:**** **. ***::. : **:*********:* ****  ** *

Barakatli       GSFPTAVEDARAYDDAARAMYGAKARVNISEQSPDANSDRTSARPNL-------------
Azamatli        GSFPTAVEPARAYDDAARAMYGAKARVNFSEQSPDANSGCTLAPPLLMSNGATAASHPS-
ABA08424.1      GSFPTAVEAARAYDDAARAMYGAKARVNFSEQSPDANSGCTLAPPLPMSNGATAASHPS-
AAL01124.1      GSFPTAVEAARAYDDAARAMYGAKARVNFSEQSPDANSGCTLAPPLPMSNGATAASHPS-
AGL08024.1      GSFPTAVEAARAYDDAARAMYGAKARVNFSEQSPDANSGCTLAPPLLTSNGATAASHPS-
AEZ68002.1      GSFPTAVEAARAYDDAARAMYGAKARVNFSEQSPDANSGCTLAPPLPTCNGATAASHPS-
BAD97369.1      GTFPTAEDAARAYDEAARAMYGALARTNFPVHPAQAPAVAVPAAIEGVVRGASASCESTT
AAY44605.1      GSFPTAVEAARAYDDAARAMYGAKARVNFSEQSPDANSGCTLAPPLPMSNGATAASHPS-
VAH57505.1      GSFPTAVEAARAYDDAARAMYGAKARVNFSEQSPDANSGCTLAPPLLTSNGATAASHPS-
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Fig. 5. AP2/ERF DNA-binding domain of DREB gene. The area underlined with a solid line shows the DNA-binding domain. The specific signal peptide sequence area is highlighted in red. The valine 14t^{h} and glutamic acid 19^{h} amino acids of the AP2 domain are shown in bold and italics. Black boxes are the aromatic rings of Trp. The specific sequence for α-helix is yellow. Green colors are the T and S residues that distinguished the Ser/Thr-rich region.

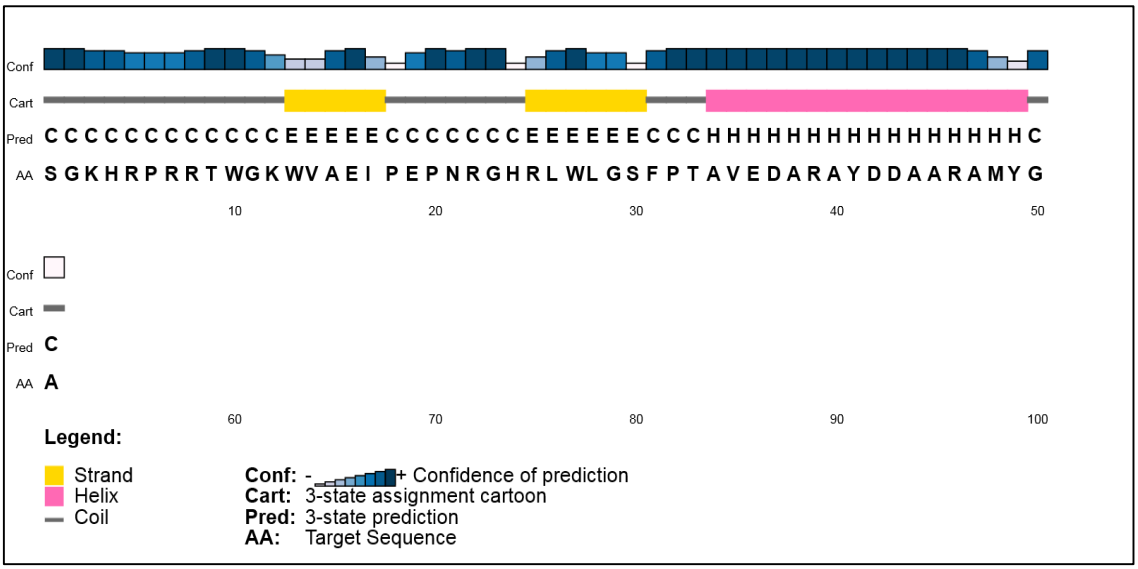

Fig. 6. Information about the secondary structure of the AP2 domain, as predicted by PSIPRED analysis.

Liu et al. (1998) have demonstrated the presence of a conserved Ser/Thr-rich region adjacent to the EREBP/AP2 binding domain containing the phosphorylation site for the regulation of gene activity. Using the protein structure prediction software PSIPRED 4.0 (Predict Secondary Structure), analysis of deduced amino acid sequences of the AP2 domain was performed in putative DREB1 gene fragments from Barakatli 95 and Azamatli 95, and one α-helix and two β-sheets (Fig 5, Fig.6) were found in the secondary structure. The 3D structure of the complex of the Arabidopsis AtERF1-DNA-binding domain and its target DNA was determined by NMR (Allen et al., 1998) and used for molecular modeling of ParCBF1. To understand the structure-function relationships, for the first time, Pandey et al. (2014) built the tertiary structure of the DREB2 protein from wheat by homology modeling based on the crystal structure of the GCC-box binding domain of *Arabidopsis thaliana*.

Protein docking with the DNA containing GCC-box revealed more similarities in the AP2/EREBP protein between *A. thailana* and *T. aestivum*. It was found that proteins interact through their β-sheet, with the major DNA groove by hydrogen and hydrophobic bond providing structural stability to the molecule (Fig. 6).

The second 300 bp DNA fragments were compared with all nucleotide sequences of plant origin collected in GenBank. This fragment shows 99% identity with certain areas on the 3B chromosome of the bread wheat (*T. aestivum* L.) genotype, 'Chinese Spring' ('CS'). Besides, it has a 46% similarity with the *gag* polyprotein of retroviruses.

To date, many studies for understanding the mechanisms and functions of DREB transcription factors have been conducted. The first DREB transcription factor, CBF1 was isolated from Arabidopsis (Liu et al., 1998). Since then, several homologs of DREB1 and DREB2 have been identified in different plants, such as barley (Choi et al., 2002, Skinner et al., 2005), canola (Jaglo-Ottosen et al., 2001), Bell pepper (Hong and Kim, 2005), soybean (Li et al., 2005), tobacco (Park et al., 2001), tomato (Jaglo-Ottosen et al., 2001) and wheat. The DREB1 gene, primarily isolated from *T. aestivum* (Shen et al., 2003), was strongly induced by drought, salinity, and low temperature. DREB2 was isolated from wheat seedlings and its expression was activated by cold, drought, salt, and exogenous ABA treatment (Egawa et al., 2006). 500 bp TaDREB DNA sequences were detected in the Iranian wheat genotypes (Andeani et al., 2009). A new DREB family member classified as TaDREB3 transcription factor was isolated by Morran et al.

(2011), who further developed some transgenic populations of wheat and barley over-expressing both TaDREB2 and TaDREB3 factors. The elevated expression in the transgenic of other CBF/DREB genes and a large number of stress-responsive LEA/COR/DHN genes, which are responsible for the protection of cells from damage and desiccation under stresses, is due to the increased expression of TaDREB2 and TaDREB3. Two isoforms of WDREB2 were isolated and molecularly characterized in wheat and WDREB2 was shown to have 3 alternative splice forms or isoforms. β isoform that lacks a transcription activation domain is inactive while α is an active isoform (Sazegari and Niazi, 2012). SNPs in the EREBP/AP2 domain of DREB1, DREB2, DREB3, DREB4, and DREB5 genes were identified and characterized in some durum wheat (*T. turgidum L. var durum*) cultivars with contrasting salt and drought tolerance (Mondini et al., 2015). The DREB gene was strongly expressed in roots followed by stem, leaf, and inflorescence (Khan et al., 2017). Interestingly, both dehydration-tolerant and dehydration-sensitive wheat varieties explored contain the DREB gene in their genome. It is supposed that DREB gene expression under normal conditions may be related to other functions in a cell (Latini et al., 2008; Khan. 2011). Identification of wheat DREB genes was performed by Niu et al. (2020) at the genome level. Functions of TaDREB genes were characterized and in total, 210 TaDREB genes, which can be divided into 6 subgroups were detected. Among them, the expression of three TaDREB3 homoeologous genes was induced by abiotic stresses. Using sequence-based phylogenetic analyses, Hassan et al. (2021) identified 32 new DREB subfamily members, not belonging to any known sub-group.

CONCLUSION

DREB transcription factor is one of the most promising candidate genes, involved in plant tolerance to multiple abiotic stresses. From this point of view, molecular and computational characterization of the DREB gene from the different wheat genotypes is important for developing new tolerant ones. We have isolated and sequenced the partial DREB1 gene from Azerbaijan's local wheat genotypes. To identify conserved regions, deduced amino-acid sequences of partial DREB1 genes and some AP2-containing proteins deposited in the GenBank, were multi-aligned. These proteins demonstrated a high level of amino acid identity, especially in the conserved regions. The gene was shown to contain a highly conserved AP2 domain, a nuclear localization signal, and a conserved Ser/Thr-rich region. The studies of transcription factors will provide important bases for plant molecular breeding. Since tolerance to abiotic stress is polygenic in nature, the transfer of any single-acting gene is likely to be insufficient to induce the desired level of tolerance. Transcription factors regulate regulon expression as a single whole gene and, therefore, can be used to simultaneously activate several downstream genes induced by stress.

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CONFLICT OF INTEREST

There is no conflict of interest in the present study.

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The effect of soil drought on malate-aspartate shuttle enzyme levels in wheat genotypes

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One of the major agricultural issues is the decrease in productivity of bread wheat varieties grown in Azerbaijan due to water scarcity caused by climate change. In this regard, drought, one of the abiotic stress factors that can directly or indirectly affect plant metabolism, has remained an active discussion topic of plant metabolism research in recent decades. The highest ratio between the variants was observed in the Murov 2 variety. As a result of studying the enzymes (AspAT and NAD-MDH) of the malate-aspartate shuttle, which plays an important role in increasing the productivity of wheat plants, it was found that the activity of both enzymes in drought-tolerant genotypes is higher compared to sensitive genotypes. AspAT, NAD-MDH, and NAD+/NADH play important roles in plant development and stress response.

Keywords: *Wheat, malate-aspartate suttle, stress, drought*

INTRODUCTION

Rapid population growth, declining biodiversity, and limited suitable and productive agricultural land pose a serious threat to meeting the food needs of the population in a globalized world (Ghatak et al., 2022, Padhan et al., 2020). In this regard, drought, one of the abiotic stress factors that can directly or indirectly affect plant metabolism, has remained an active topic of discussion in recent decades of research in this area (Chen et al., 2019; Kapoor et al., 2020). The enzymes aspartate aminotransferase (AspAT/ASAT/AAT, EC 2.6.1.1) and NAD-malate dehydrogenase (L-malate-NAD-oxidoreductase, NAD-MDH, EC 1.1.1.37) the primary enzyme in the malate–aspartate shuttle. provide a connection between carbohydrate metabolism and amino acid metabolism, playing an important role in both processes of catabolism and anabolism. The enzyme malate dehydrogenase, which catalyzes the mutual conversion of malate and oxaloacetate, is found in all living organisms and subcellular organelles (mitochondria, glyoxysomes, peroxisomes, chloroplasts) (Liszka et al., 2020). AspAT mediates the transport of α-amine groups between aspartate and glutamate. It catalyzes the reversible reaction converting aspartate and α-ketoglutarate to oxaloacetate and L-glutamate. Aspartate is an intermediate metabolite of amino acid metabolism and the Krebs cycle. It is involved in the growth and development of plants and in the formation of the mechanism of stress tolerance (Han et al., 2021; Jia et al., 2016; Ullah et al., 2017; Gargallo-Garriga et al., 2018; Khan et al., 2019; Barickman et al., 2020; Zhang et al., 2017). The main role of aspartate in eukaryotic cells is to transport reducing equivalents synthesized as a result of glycolysis across the mitochondrial membrane and to participate in the generation of ATP (Borst, 2020). Aspartate synthesized in mitochondria is transported to the cytosol using special transporters. Aspartate in the cytosol is converted back to malate by AspAT and MDH (Singh et al., 2022), resulting in an increase in the NAD+/NADH ratio (Easlon et al., 2008, Borst, 2020). The NAD+/NADH ratio integrates many

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aspects of metabolism and plays an important role in plant development and stress response. A detailed study and investigation of aspartate anabolism and catabolism and associated pathways (i.e. Asp family amino acids, nucleotides, NAD, Krebs cycle and glycolysis) is extremely important to expand our knowledge of cell division, growth, and self-renewal.

During the daytime, malate is accumulated in leaf cells, particularly, in the vacuole, whereas the cytosolic malate concentration is maintained at a relatively constant, low level (Gerhardt et al., 1987). Some cytosolic malate is imported into the peroxisomes, where it is used by peroxisomal NAD-MDH to recycle OAA and regenerate the NADH required for hydropyruvate reduction (Selinski and Scheibe, 2019). In mitochondria, both the malate-OAA shuttle (Kramer and Evans, 2011; Vishwakarma et al., 2015; Yoshida et al., 2007; Noguchi and Yoshida, 2008; Zakhartsev et al. 2016; Zhao et al. 2018; Pastore 2007) and photorespiration (Gardeström and Wigge, 1988; Igamberdiev et al., 2001) have been proposed to provide reducing equivalents to the mitochondrial electron transport chain (mETC) for ATP production. In a flux balance model of the mature leaves of C3 plants, both photorespiration and the malate OAA shuttle are predicted to contribute to feeding NADH into the mETC (Cheung et al., 2014). Experimental data obtained from barley leaf protoplasts (Gardeström and Wigge 1988; Igamberdiev et al., 2001; Gardeström and Igamberdiev 2016) and isolated mitochondria (Lee et al., 2010) suggest that photorespiration is the major source of reducing equivalents to the mETC. However, this has not been examined at a whole plant level, and the direction of the flow of reducing equivalents between different subcellular compartments during photosynthesis has not yet been fully resolved (Shameer et al., 2019). Here, by employing NADPH and NADH/NAD+ sensors, we examined plant dynamic changes in the NADPH pools and NADH/NAD+ ratio in the stroma and cytosol upon illumination.

The study aimed to look into the role of malate-aspartate shuttle enzymes (AspAT and NAD-MDH) in increasing the productivity of wheat.

MATERIALS AND METHODS

Four wheat genotypes from the gene bank of the Research Institute of Crop Husbandry of the Ministry of Agriculture were chosen for the study: Murov 2, Aran, Gyzyl Bugda, and Zirva 85. The bread wheat genotypes were grown in a laboratory with an artificial climate under a photoperiod of 16h/8 h and a temperature of 240C/180C, day/night mode, respectively, and a relative humidity of 50%. 14-day-old seedlings were subjected to drought stress. The measurements were carried out in two variants, 10 biological and 3 technical replicates.

To determine the enzymatic activity, the leaves were washed with distilled water, dried on filter paper, and crushed for 3 minutes in a mortar using 100 mM Tris-HCl (pH 7.8) buffer containing 5 mM DTT, 5 mM $MgCl_2 \cdot 6H_2O$, 1 mM EDTA ·4Na, 0.5% Triton X-100 and 1% PVP. After filtering the resulting homogenate, the filtrate was first centrifuged at 1000 g for 10 minutes and then at 5000 g for 30 minutes. This process was carried out at a temperature of +4°C. The supernatant was used to determine the activity of the enzymes.

NAD-MDH activity was determined by the spectrophotometric method (Ultrospec 3300 pro, Amersham, USA) (Scheibe and Stitt, 1988). The reaction medium consisted of 100 mM Tris-HCl (pH 8.0) buffer containing 1 mM oxaloacetate, 10 mg/ml bovine serum albumin (BSA), 10 mM MgCl2, 0.15 μM NADH and 5-10 μl of the enzyme preparation. The reaction was initiated by adding 1 mM oxaloacetate to the medium. The medium for the direct reaction consisted of 100 mM Tris-HCl (pH 9.0), 30 mM malate, 0.2 mM NAD. Measurements were carried out in spectrophotometric cuvettes with a volume of 1.0 ml. The amount of NADH was determined by the decrease in the optical density of the molar concentration of this compound at a wavelength of 340 nm for 1 minute.

The reaction medium for determining aspartate aminotransferase activity consisted of 100 mM HEPES-KOH (pH 7.4) and 100 mM Tris-HCl (pH 8.5), 2 mM EDTA, 2.5 mM 2-oxoglutarate, 10 μg/ml pyridoxal phosphate, 10 mM DTT, 12 U/ml MDH, and 0.2 mM NADH. The reaction was initiated by adding 20 μl of leaf

extract and 2.5 mM L-aspartate to the medium (Alfonso and Brüggemann 2012).

Total soluble protein was determined using 0.12% Coomassie Brilliant Blue G-250 solution. Optical density measurements were carried out using a spectrophotometer (Ultrospec 3300 pro, Amersham) at a wavelength of 610 nm. Bovine serum albumin was used to construct the calibration curve (Sedmak and Grossberg, 1977).

All experiments were performed in triplicate and errors were determined using the Student's t-test statistical analysis program. Differences between mean values were considered significant at P values <0.01, 0.005.

RESULTS AND DISCUSSION

The activity of NAD-malate dehydrogenase was determined in the leaves of bread wheat varieties grown under artificial climate conditions. NAD-MDH activity in the leaves of the Zirva 85 variety increased 1.5 times under stress (17.38±2.0 µmol OA/protein min^{-1}) compared to samples grown under normal watering (11.4±1.2 µmol OA /protein min^{-1}). The activity of the NAD-MDH enzyme in the varieties Aran and Murov 2 was close to that of the variety Zirva 85 (Figure 1).

Unlike other varieties, in the leaves of the Gyzyl Bugda variety, the activity of the NAD-MDH enzyme decreased by 1.2 times (17.6 ± 1.8 µmol OA/protein min^{-1}) under stress conditions compared to samples (15.0 ±1.45 µmol OA/protein min^{-1}) grown under normal watering. The highest NAD-MDH enzyme activity was observed in leaves of the Aran variety (18.08 ± 2.0 µmol OA/protein min^{-1}) under stress (Figure 2). Malate is involved in many physiological processes, such as providing NADH for the nitrate reduction reaction, fatty acid biosynthesis for the carbon chain and photorespiration, stomatal movement through regulation of osmotic pressure, control of cellular pH, redox hemostasis, as well as transport and exchange of reduced equivalents between cellular compartments. The synthesis of malate is the result of the sequential action of PEPC and MDH. In this work, we studied the activity of the enzyme aspartate aminotransferase in flag leaves of wheat varieties (Gurbanova et al., 2021).

The activity of the AspAT enzyme was higher in stress variants of all studied wheat varieties compared to plants grown under normal watering. The activity of the AspAT enzyme increased 2 times in leaf samples of the Zirva 85 variety under stress conditions (0.116±0.02 µmol mg^{-1} protein min^{-1}) compared to the watering variant (0.059±0.007 µmol mg^{-1} protein min^{-1}). Among the studied varieties of bread wheat, the highest indicator was found in the Zirva 85 variety grown under drought conditions. The enzyme activity in the Aran variety under drought (0.056±0.007 µmol mg^{-1} protein min^{-1}) was slightly higher compared to the control plants (0.054±0.008 µmol mg^{-1} protein min^{-1}).

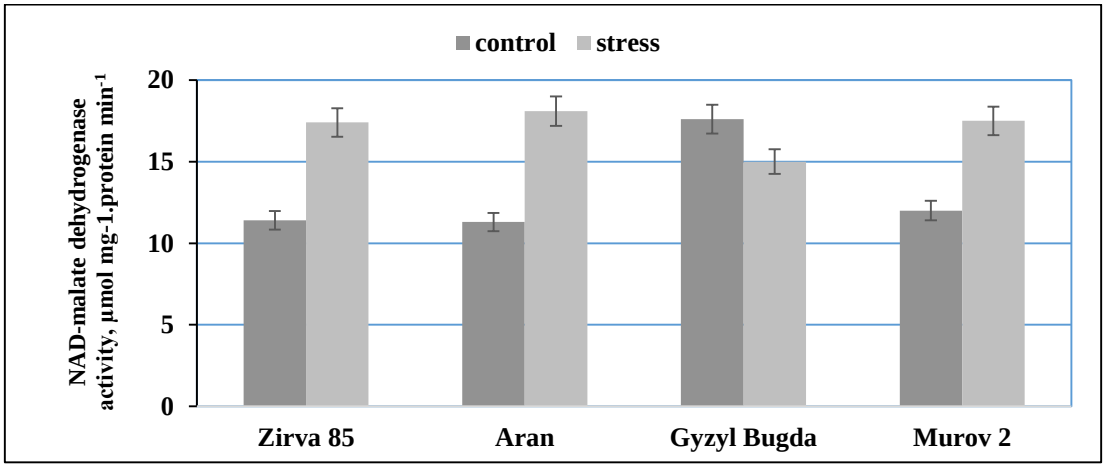

Fig. 1. Changes in NAD-malate dehydrogenase activity in leaves of bread wheat varieties grown under artificial climate conditions

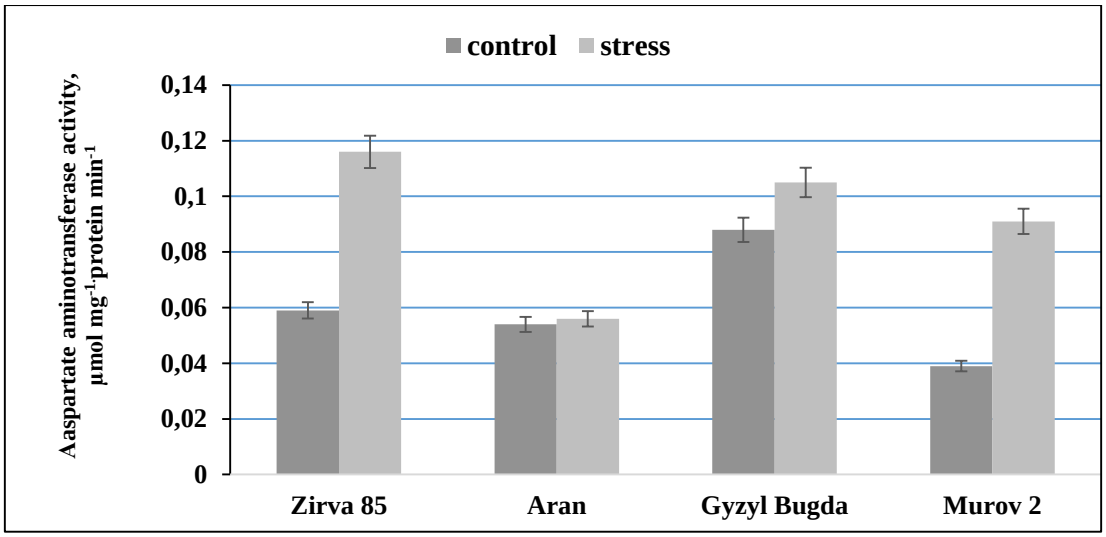

Fig. 2. Changes in aspartate aminotransferase activity in leaves of bread wheat varieties grown under artificial climate conditions.

The activity of the AspAT enzyme in the control and experimental variants was, respectively, 0.088±0.01 µmol mg^{-1}.protein min^{-1} and 0.105±0.001 µmol mg-1.protein min-1. In this variety, the activity of the AspAT enzyme in the experimental variant was two times higher than the value of the control variant. The activity of the AspAT enzyme was 2.3 times higher in plants of the Murov 2 variety (0.091±0.001 µmol mg^{-1}protein min^{-1}) under drought compared to the control plants (0.039 µmol mg^{-1}.protein min^{-1}). The highest ratio between the variants was observed in the Murov 2 variety. As a result of studying the enzymes (AspAT and NAD-MDH) of the malate-aspartate shuttle, which plays an important role in increasing the productivity of wheat plants, it was found that the activity of both enzymes in drought-tolerant genotypes is higher compared to sensitive genotypes. AspAT, NAD-MDH, and NAD^+/NADH play important roles in plant development and stress response. Lim and colleagues suggest that, at the light intensities we used, photorespiration supplies a large amount of reducing equivalents to mitochondria during photosynthesis, which exceeds the NADH dissipating capacity of the mETC. Consequently, the surplus NADH must be exported from the mitochondria to the cytosol through the mitochondrial malate-OAA shuttle (Lim et al., 2020).

The malate shuttle, which contributes to photorespiration at multiple levels, is involved in the transamination of glyoxylate into glycine coupled with the conversion of glutamate to 2-oxoglutarate in the peroxisome. This reaction is catalyzed by glutamate:glyoxylate aminotransferase (GGT). The plastidial malate/2-oxoglutarate shuttle transports glutamate from the chloroplast, which acts as an NH3 donor for glyoxylate transamination. According to the results obtained, the studied enzymes of the malate-aspartate shuttle play an important role in the adaptation processes of higher plants - the distribution of carbon and energy.

CONFLICT OF INTEREST

There is no conflict of interest in the present study.

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Disease-associated variations of autosomal STR loci: minireview

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The human genome is constantly undergoing various types of mutations. Some of them seem to be inherited, while others occur spontaneously under the influence of external environmental factors. Some diseases are caused by mutations that are inherited from the parents. Currently, the investigation of new reliable markers for the prediagnosis of hereditary diseases is one of the urgent issues. Several of these markers are identified by genome-wide association studies (GWAS), others by candidate gene approach (CGA), as well as by individual experimental studies. The current article provides a summary of research related to the study of the association of STR markers that are included in human identification kits with various diseases. The key idea of this article was to highlight the alleles as well as several trisomies that are associated with Down's and Edwards' syndromes located in the coding region, mainly variants of loci (CSF1PO, TPOX, THO1, vWA, FGA, etc.) inside the genes, which are associated with diseases. Based on the experimentally obtained results these markers can serve as additional diagnostic tools. Moreover, these markers can be used in family planning policy.

Keywords: *STR loci, mutation, genome instability, trisomy, loss of alleles, disease-associated alleles, cancer, Down syndrome, Edward syndrome*

From fertilization to birth, from birth to death, the human genome undergoes changes as a result of mutations that occur continuously throughout life. Some of these mutations are neutral, while others are aggressive or harmful. Excessively aggressive or harmful mutations cause the death of the carriers, or carriers suffer from various pathologies. Compared to normal variations, some of the pathogenic variations are eliminated directly, while others did not transmitte to future generations. In comparison to normal (neutral) variations, pathogenic variations are less widespread and can rarely consolidate their place in the population either randomly or transiently. A previous study conducted by Mustafayev et al. revealed the mutations that were detected during paternity tests in the Azerbaijani population (Mustafayev et al., 2019). In this review, the analysis of studies related to the detection of associative relationships of specific alleles/variations of STR loci with various diseases in different populations was shown.

One of the main reasons of changes is dynamic mutations, and it is important to study the mechanisms of formation, nature and role of all fixed variations that are presented in the genome in the form of repeated sequences (Pearson et al., 2005). In general, the instability and polymorphisms of the genomes is a universal phenomenon. According to the extent of occurrence, two types of genome instability were distinguished:

1) Chromosome instability (CI) - mainly cancer cells, extra chromosomes and chromosome losses creating an abnormal karyotype, all kinds of chromosomal

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rearrangements, etc. This type of instability can be inherited during cell divisions and continuously presented as new variations in further generations.

2) Microsatellite instability (MI) is polymorphic pathogenic changes occurring directly in different types of microsatellite DNA sequences.

Currently observed variations are mostly normal ones. The examples of common normal variations at the chromosomal level are reported below:

1) The size of the heterochromatin regions at the centromeres of chromosomes 1, 9, 12, and 16 and the long arm of the Y-chromosome can change;
2) The short arms of acrocentric chromosomes 12, 13, 14, 15, 21, and 22, whose centromeres are located very close to one of the ends of the arms, showed significant variability in size. Usually, the so-called satellite part, which is connected to the main part of the chromosome with a thin short "thread", can be observed in the most distal (farthest) area.
3) While cells are cultured under conditions that make DNA replication difficult (for example, lack of thymidine or addition of DNA-polymerase inhibitor aphidicolin), the variability of "fragile chromosome" regions (fragile sites) becomes evident (uncoiled chromatin pieces). Most of the fragile chromosomal regions are normal variations, except for a few ones (for example, the fragile sites FraXE and FraXF that are directly linked to mental retardation are pathogenic).

Genome variations occur due to changes in the number of copies of tandem repeat sequences, full or partial insertions/deletions and point mutations (SNP). Recently, 325 (>400) million SNPs have been detected in the human genome using the array-comparative genomic hybridization (ACGH) method, of which 15 million have a frequency of more than 1% among world populations. The HapMap project (2003, 2010) reported >1447 regions with high variability within the sequences of at least 1 Kb in length in the human genome. In total, these regions possessed with the length of ~360 Mb and cover ~12% of the genome. The average size of highly variable segments was 250 Kb. Short variations were identified using the paired-end mapping technique and appeared to be more common in the genome (Korbel et al., 2007).

SNPs appeared in both normal and pathogenic changes due to their ability to occur everywhere in the genome – in coding and non-coding elements, as well as in all types of repeats via all possible ways (insertions, deletions, substitutions, trans- and inversions, etc.).

Microsatellites, known as short tandem repeats (STRs) and consisting of 2–6 bp in their core sequences, are small-size DNA sequences with multiple repeats that make up approximately 3% of the human genome (Lander et al., 2001; STRBase (SRD-130), 2023). Repeated core units of polymorphic STR loci, that located mainly in non-coding regions of the genome and vary widely (Butler, 2006; Biscotti et al., 2015). However, there are STRs localized in intron and promoter regions. STRs located in promoter regions are associated with transcription regulatory elements and participate in transactivation (Sawaya et al., 2012; Chen et al., 2016). Possessing with high level of polymorphism, makes these markers like SNP and STR valuable in solving various problems. Examples of such areas of application include interspecies and intraspecies differentiation studies (Deniskova et al., 2016), prenatal clinical studies (Agarwal et al., 2014; Li et al., 2021), preimplantation screening of β-thalassemia (Sharifi et al., 2019), in the determination of kinship relationships at the level of sister and brother (Chakraborty, 2016; Tang et al., 2012), in controversial maternity/paternity and human identification tests (Pinto et al., 2013; Lee et al., 2015; Ramsos and Valloni, 2015), in forensic and population studies (Anghel et al., 2015, Eskandarion et al., 2015; Gurkan et al., 2015; Huang et al., 2015; Tan et al., 2017; He et al., 2018a, 2018b; Amirian et al., 2019; Anwar et al., 2019, Khubrani et al., 2019; Nowroski et al., 2019; Aalbes et al., 2020; Kakkar et al., 2020; Kumawat et al., 2020; Mammadov et al., 2020; Pilav et al., 2020; Sahoo et al., 2020; Badiye et al., 2021), in sequence-based assignments (Tao et al., 2021), etc.

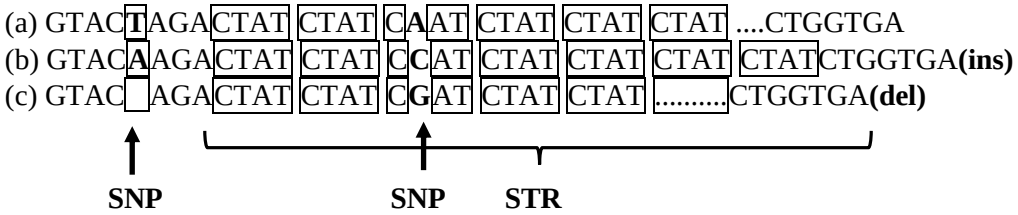

Figure. Schematic representation of genetic variations in DNA sequence (Jobling and Tyler-Smith, 1995).

By slightly modifying the scheme proposed by M.A.Jobling and colleagues (Jobling and Tyler-Smith, 1995; Jobling, 2004; Jobling et al., 2014), genetic variations occurring outside and inside of STR loci with the participation of Ins/Del mutations as well as single nucleotide substitutions can be schematically visualized as shown in the Figure. Internal SNP mutations lead to the formation of isoallelic forms (with no change in the number of nucleotides of the allele, but a difference in composition). These mutations located in non-coding regions (including silent mutations in coding regions) are neutral or normal since they are not revealed in terms of function, like in STRs, which are structural elements of genes, either internal or external SNPs, ins/del-mutations that could be pathogenic and associated with several diseases.

The study of the association of some alleles of the STR loci with certain diseases is controversial. This is mainly used to find their associations with any disease, as well as using them for identification, disputed paternity/maternity, and other tests including doubtful results of genetic tests. Another issue is the ethical problem, like preserving confidentiality while detecting an allele of any STR locus that is associated with a certain disease in the genotype of any individual. In case, if the detected allele is associated with a harmful disease, the aspects of public communication and notification to the tested person should be investigated. However, by assuming that one of the promising approaches is the investigation of disease-carrying gene linkage through disease-related alleles of STR loci, it is appropriate to use such STR loci in practice (Ghebranious et al., 2003). The promising advantage of such an opportunity was previously expressed by K. Kimpton et al. (Kimpton et al., 1995), and later supported by the European DNA-Profiling Group. Below the alleles that were found to be associated with diseases at some of the loci and used in human identification were reported.

The THO1 STR locus is a tetrameric STR locus located in intron 1 of the tyrosine hydroxylase gene. Tyrosine hydroxylase catalyzes the hydroxylation of L-tyrosine to L-DOPA and is the rate-limiting enzyme in the synthesis of catecholamines such as noradrenaline or adrenaline, which play a key role in blood pressure regulation. In a clinical study, a strong correlation was observed between the 9.3 and 10 alleles and essential hypertension (Sharma et al., 1998). F. Rao et al. (2010) revealed that the widespread variation in the proximal promoter of the TH gene has functional and cardiovascular risk-related effects. There are other reports with similar associations (Klintschar et al., 2004; 2005). A study by R.Szibor et al. (2005) suggested excluding such STR markers from the target set during the evaluation of identification tests.

The X-chromosomal STR locus HumARA (Edwards et al., 1992) is a CAG repeat in exon 1, the coding region of the androgen receptor gene, which was associated with several genetic diseases (see: Szibor et al., 2005). Among the 18 core loci most commonly used in human identity testing, this locus is the only one that is located in a gene coding region (eg, exon), thus may have a "probability" of causing genetic defects (Tan and Lai, 2005).

Indeed, loss of heterozygous or allelic disbalance of a number of key STR loci described in many studies was reported to be useful in monitoring various genetic diseases. For example, the D8S1179 STR locus was used for the determination of localization of a gene that is associated with Meckel-Gruber syndrome, the most common monogenic cause of neural tube defects (Morgan et al., 2002).

The main reason for doubting the association of several blind STR loci with certain diseases in the most of this kind of studies is the use of genome-wide screening. For example, there is a Marshfield panel (Weber set 10) that scans over 400 STRs in the human genome, including TPOX, D7S820, D8S1179, D13S317, D16S539, and D19S433 (Ghebranious et al., 2003). Therefore, the existence of association between alleles of THO1, which is known for its negative reputation, in a number of patients with schizophrenia (Meloni et al., 1995a, Thibaut et al., 1997) and bipolar disorder (Meloni et al., 1995b), was reported. However, it is known that other researchers did not confirm these associations (Burgert et al., 1998, McQuillin et al., 1999). Recent studies have shown that individuals carrying the 7 allele of THO1 were less nicotine dependent, although these data are not definitively confirmed (Anney et al., 2004).

F.Gao and colleagues (Gao et al., 2013) studied the association of microsatellite STR loci polymorphism (ATCC)n1, D1S1621, and (ATCC)n2 located in introns 1, 8, and 9 of the DISC1 (Disrupted-in-schizophrenia-1) gene with the risk of developing of the schizophrenia in a Chinese Han population. The study was conducted on 310 schizophrenic patients and 400 controls. It was found that the frequency of alleles 12 of (ATCC)n1, 11 and 12, 13 and 15 of D1S1621, and 10 of (ATCC)n2 was significantly higher in schizophrenic patients than in controls, in comparison to alleles 9 and 10 of (ATCC)n1, and alleles 16, 17 and 18 of D1S1621.

Trisomy-21, known as Down syndrome, can be characterized by the presence of three alleles at any polymorphic marker on chromosome 21 (Liou et al., 2004). STR locus D21S11 is a more correct test to identify the trisomy-21 (Pertl et al., 1994). In addition, evaluation of trisomy-18 (Edwards syndrome) in prenatal samples was performed with the D18S51 marker (Yoon et al., 2002). Moreover, loss of heterozygosity (for any reason, an allele located on one of the homologous chromosomes becomes a "null" allele, causing highly homozygous genotype(s)) or a high level of allelic imbalance (for the same reason, causing multiallelic genotype(s)) in some cases was assumed to be associated with cancer (Rubocki et al., 2000).

In 2021 K.Wang et al. (2021) reported a rapid prenatal diagnosis method of Down (trisomy 21) and Edward (trisomy 18) syndromes by amplification of multiplet STR loci via designed fluorescent-labeled primers (STR-FQ-PCR). The non-invasive prenatal diagnostic technology (NIPT) method was tested on 64 amniotic fluid samples for the presence of trisomies 18 and 21, and the results were compared with karyotype and chromosomal copy number variation (CNV) analysis. The aneuploidy test gave a positive result in 61 samples, 14 of which showed Edward's syndrome, and 47 showed Down's syndrome. In total 460 STR locus genotypes were detected, of which 84 related to Edward's syndrome and 376 to Down's syndrome. Chromosomal karyotype analysis showed that all detected samples were chromosomal aneuploidy - 15 of them with trisomy 18, including 14 homozygous and 1 chimeric type, 49 with trisomy 21, including 47 homozygous and 2 chimeric type. CNV analysis revealed 62 cases of chromosomal aneuploidy, 14 of which were trisomy 18 and 48 for trisomy 21. The authors concluded that the detection accuracy rate of STR-FQ-PCR technology was 95.31%, and for karyotype analysis that was 100%. For non-chimera and non-structural abnormal samples, the results of karyotype analyses and that of STR-FQ-PCR technology were 100% identical.

S.F. Alharbi et al. (2022) amplified STR loci with specific primers in 15 chronic myeloid leukemia patients and 15 healthy individuals and showed that alleles 9 and 9.3 of the tyrosine hydroxylase 1 (THO1) STR marker were more frequently detected in leukemia patients.

STR loci also showed instability in other forms of cancer. This is mainly related to DNA repair systems. For example, such associations were found in lung cancer (Zhang et al., 2018), lung and liver cancer (Qi et al., 2018), gastric cancer (Hui et al., 2014), in papillary thyroid cancer (Dang et al., 2020), in esophageal cancer (Kaifi et al., 207), and in leukemia (Filoglu et al., 2014; Bawazir et al., 2019).

Z.L. Wang et al. (2012) detected the association between allele frequency of 15 autosomal STR loci included in the AmpFLSTR™ Identifiler™ PCR Amplification Kit marker set and chronic myeloid leukemia

(CML). The study was conducted on 745 healthy subjects and 132 patients with CML. Comparison of allele frequencies between the patient and healthy groups revealed statistically significant differences ($P<0.05$) of three STR markers, CSF1PO, vWA and TPOX.

H.Dasnhow and colleagues (2018) proposed a new method called STRetch, which allowed the detection of all pathogenicity-causing STR expansions in the genome, and allowed finding new ones. The method was based on information obtained from genome-wide short reads (sequencing) of known and novel pathogenic loci. The STRetch is the source-available software (github.com/Oshlack/STRetch).

X.Qi and colleagues (2018) conducted a study to analyze the possibility of screening lung and liver cancer susceptibility by using genetic markers rather than genes that are directly associated with the disease. The study revealed statistically significant associations of allele 20 of D18S51 with lung cancer, as well as allele 30.2 of D21S11 and allele 18 of D6S1043 STR marker with liver cancer. These analyses showed that STR markers that are included in the CODIS system can predict susceptibility to cancer.

A study by L.Hiu et al. (2014) revealed that among young individuals with gastric cancer, allele 23 of D2S1338 and allele 11 of D6S1043, as well as allele 16 of D8S1179 and allele 13 of D5S818 showed higher frequency in pairs.

N. Wyner and colleagues (2020) reviewed 107 articles on the association of forensic STRs with phenotype and found 24 markers for 50 unique traits that are related to that in 57 articles. The THO1 marker was associated with 40 different genotypes for 27 traits, five of which showed that THO1 was associated with schizophrenia. Although none of these traits were directly independent causes or predictors of the disease, the statistical significance of the association was nevertheless high.

N.von Wurmb-Schwark and colleagues (2011) conducted a study related to the genetic association of the THO1 STR marker that showed the tyrosine hydroxylase 1 (THO1) gene as a candidate gene for human longevity in the Italian population. In this article, 471 elderly (97-110 years old) and 462 young controls (19-75 years old) living in Germany were studied, but the expected association was not detected. Nevertheless, the allele frequencies between the studied groups and the previously published study were consistent. However, significant differences in the frequencies of the THO1 allele 9.3 were observed between Germans and Italians, which confirmed the fact that the frequency of this allele decreased in the West-East and North-South directions throughout Europe.

Based on the experimental results, S.Alam and colleagues (2011) hypothesized that the 9th allele of the THO1 STR locus is associated with susceptibility to malaria (*Plasmodia falciparum*). Note that the THO1 microsatellite locus is located in the human immunoregulatory region and close to the β-globin gene.

M.A.Meraz-Ríos et al. (2014) studied the association of STR loci with venous thromboembolism (VTE) disease in 177 patients and 531 healthy controls. The study showed that allele 18 of the vWA microsatellite of the von Willebrand factor α-fibrinogen gene as well as alleles 9 and 12 of the thyroid peroxidase gene TPOX microsatellite were significantly associated with VTE disease. In addition, this risk was higher in individuals with both vWA-18/TPOX-12 (95%CI, OR=1.02-3.64) and vWA-18/TPOX-9 (95%CI, OR=4.93-21.49).

G.Sutherland and colleagues (2008) performed haplotype analysis of the IGF2-INS-TH gene cluster and revealed that the haplotype of IGF2-rs680, INS-rs689 SNPs and the TH-6 allele were not significantly presented in idiopathic Parkinson patients (OR=0.42, 95% CI , 0.25-0.72, P=0.001).

In addition, several studies showed the statistically significant association of the allele 9.3 of the THO1 STR locus with sudden infant death syndrome (SIDS) (Klintschar M, 2008; Courts C, Madea B, 2011).

Chun Yang et al. (2022) studied the association of 20 autosomal STR loci with the schizophrenia disease. The study was conducted on 355 schizophrenics and 473 healthy males. Although statistical differences were found in the distribution of genotypes and alleles of D13S317, D5S818 loci between two groups, and no difference was found in the remaining 18 STR loci. Univariate analysis showed that there were statistically significant differences in the

distribution of the (10, 11) genotype and allele 11 of the D13S317 locus and the (7, 10) genotype and allele 7 of the D5S818 locus (P<<0.005 compared to the control in both cases) between two experimental groups. Based on the obtained results it could be assumed that the above-mentioned genotypes and alleles of D13S317 and D5S818 STR loci were associated with the risk of developing schizophrenia in males.

Longevity is a complex and multifactorial phenomenon, determined by genetic, epigenetic, environmental, stochastic, and other factors. The main purpose of the research conducted by N.G. Bediaga et al. (2015) was to show the statistically relevant association between polymorphism of hypervariable STR loci such as HUMTHO1 (THO1) and HUMCSF1PO (CSF1PO) that are used in forensic practice and longevity. In a way to study 21 autosomal STR loci polymorphisms, 304 people aged 90 and over and 516 younger controls of European origin living in northern Spain were studied. The study confirmed the previously obtained results of the THO1 and CSF1PO STR loci. In addition, there were significant differences in the distribution of alleles for a total of 6 STR loci, of which the D12S391, D22S1045, and D2S441 STR loci were also significantly associated with longevity. This can be explained by the fact that the genetic pattern of longevity is more complex and depends on multiple genetic factors.

Profiling of disease-associated STR loci is associated with several challenges related to the length of reads, which affect the accuracy of the results. This limitation is mainly due to the generation of repeated reads (especially in loci consisting entirely of C and G). To partially overcome these limitations, H.Tang and colleagues (Tang et al., 2017) performed a sequencing-based whole genome profiling analysis of disease-associated alleles of hypervariable STR loci in 12,632 individuals. For this purpose, the authors improved the existing TREDPARSE software package for 30 known diseases and showed that this program is superior to any other program.

Study by N.A.Al.Sharhan et al. (2022) revealed the evaluation of loss of heterozygosity and microsatellite instability (MI) in circulating extracellular DNA (exDNA) in individuals with breast cancer using human identification STR markers (AmpFlSTR MiniFiler Human Identification Kit). The study was conducted on 41 patients and 40 healthy women. As a result of the study of the DNA profiles of patients and controls, statistically significant differences were reported in the frequencies of allele 8 of the D7S820 locus, alleles 29, 30.2 and 32.2 of the D21S11 locus, and allele11 of the CSF1PO locus, as well as the loss of heterozygosity in the profiles. The study showed that the application of exDNA microsatellite instability in early diagnosis of breast cancer can provide effective results.

In order to assess the integral stability and degradation rate of tumor-specific genomic DNA, E.E.Nikulina and colleagues (2022) performed STR loci profiling of DNA isolated from plasmacytomas of archived materials. The study was conducted on 10 patients (7 women, 3 men, average age 53.5) who were treated for advanced plasmacytoma multiple myeloma (MM) in 2013-2021. As a result of the study, 4 out of 10 people were observed for loss of heterozygosity (HL) caused by duplication or deletion of one of the two alleles on chromosomes 1 (1q42), 6 (6q14), 7 (7q21.11), 13 (13q31.1) and 21 (21q21.1).

It is known that a three-allelic pattern was observed in the genotyping of STR loci during several diseases (e.g. Down's syndrome). X.Y.Ma and colleagues (Ma et al., 2023) revealed the abnormal triallelic patterns that create difficulties and uncertainties in assessing the accuracy of the results of actual forensics cases performed with autosomal STR loci. The article also reviewed the types, formation mechanisms, frequency of occurrence, genetic pattern, and quantification of triallelic patterns in autosomal STR (Ma et al., 2023).

C.Lei and colleagues (Lei et al., 2023) for the first time proposed a new system for the detection of aneuploidy and its erroneous chromosomal origin, which caused spontaneous abortions during 2018-2020. Compared to low-pass G-binding karyotyping, the proposed system increased the detection rate of chromosomal abnormalities in 500 unexplained recurrent spontaneous abortions to 56.4%. In this study, a total of 386 STR loci located on twenty-two autosomes and two sex chromosomes (X and Y chromosomes) were developed, which can help

distinguish triploidy, uniparental diploidy, and maternal cell contamination, as well as determine the parental origin of extra chromosomes. It was not possible to perform this for the cases of miscarriages with existing methods. Moreover, it was revealed that among the errors testing for aneuploidy, trisomy was the most frequently detected error (33.4% overall, and 59.9% in the extra chromosome group). In trisomy samples, 94.7% of extra chromosomes were of maternal origin, and 5.31% were of paternal origin. The proposed new system improved the method of genetic analysis of miscarriages by providing additional reference information for the clinical management of pregnancy (Lei et al., 2023).

Recently, most STR loci were considered "junk" DNA since they were located in non-coding regions of the genome and were not able to express any phenotype. As was mentioned above in some populations STR markers that are used in forensic practice were associated with a number of diseases. Chinese scientists J.Yang and colleagues (2022) examined the association between three facial characteristics (single or double eyelid, with or without epicanthus, unattached or attached earlobe) and 15 STR loci in 721 unrelated Han individuals. In order to predict the presence of phenotypic relationship, additionally 1,993 unrelated individuals were included in the study, and STR and geographic data of 27,199 individuals whose results were available in the literature were collected. During the analysis, the correlation between facial characteristics and STR markers was not observed. Although the results were statistically significant for alleles at only two STR loci, allele 19 of D2S1338 and allele 18 of FGA (statistical confidence after Bonferroni correction P=0.0032, P=0.0030, respectively), the predictive validity was low. Principal component analysis for STR and biogeographic data showed that the first three components could explain 87.7% of the variation, but the prediction accuracy was only 25.2%.

CONCLUSIONS

In all these reviewed studies, some microsatellite STR markers, mainly used in forensic medicine to solve identification issues of genetic examinations in criminal and civil cases have an association with cancer, schizophrenia, hypertension, muscular dystrophy, Edwards' and Down's syndromes, cardiovascular and other diseases. The loss of alleles and the difference in frequency of alleles between sick and healthy were frequently observed in analyzed scientific studied. In addition, many studies were conducted to study the association of STR markers that are currently used in forensic medicine practice with diseases such as different types of cancer, predisposition to schizophrenia, etc as well as in their early diagnosis. In conclusion, despite the fact that in different populations various and contradictory results were obtained, the use of STR markers in disease identification is a relevant and promising tool.

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Algoflora of the northern littoral part of the Azerbaijani sector of the Caspian Sea (Siyazan and Shabran districts)

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Although studies on the algoflora of the Azerbaijani sector of the Caspian Sea have been conducted since the 60s of the 20th century, very little information is available about the diversity and distribution areals of algae in this sea. The paper presents the results of the study of the diversity, taxonomic structure, and ecological characteristics of marine macrophytes and microscopic algal flora of the Caspian Sea shores in the northeastern part of the Azerbaijan Republic (the territory of Siyazan and Shabran regions). 28 species of algae belonging to 4 divisions, 14 orders, 16 families, and 21 genera were identified in the research area. Their belonging to divisions *Bacillariophyta* (13 species), *Chlorophyta* (6 species), *Cyanophyta* (5 species), and *Charophyta* (4 species) was established. The *Bacillariophyta* division is the dominant group in the composition of the studied algal flora with 46.4%, *Chlorophyta* constitutes 21.4%, *Cyanophyta* -18%, and *Charophyta*-4.2%. *Naviculaceae, Oscillatoriaceae, Spirogyraceae,* and *Cladophoraceae* were found to be the leading families in algal flora. Out of 28 algae species detected, 22 species were found to be new to the research area.

Keywords: *Agoflora, ecological groups, algae, taxonomic analysis, ecological analysis*

INTRODUCTION

Using light energy, algae that are a significant part of the photoautotrophic organisms of the aquatic environment, perform the biosynthesis of cell components, including those involved in energy exchange for their reproduction. Algae are mainly typical aquatic organisms[1], distributed in fresh and salt water basins and hot springs. They include macrophyte and microscopic algae (microalgae), which are representatives of green (*Chlorophyta*), blue-green (*Cyanophyta*), yellow-green (*Xanthophyta*), red (*Rhodophyta*), diatom (*Bacillaryphyta*), golden (*Chrysophyta*), and brown (*Ochrophyta*) algae divisions (Pienaar and Pieterse, 1990). Representatives of all divisions are eukaryotes, except cyanobacteria, which are prokaryotes. Algae actively participate in the cycle of sulfur, nitrogen, carbon, and other biogenic elements. They are also primary producers of organic matter in aquatic ecosystems. Algae synthesize proteins, lipids, and carbohydrates, valuable biologically active substances, and are rich in mineral elements. Due to their photosynthesizing abilities, they play an important role as one of the main sources of oxygen in the atmosphere. This, in turn, draws attention to the study of the issues of biology, ecology, systematics, physiology, and biochemistry of algae (Vozjinskaya and Kamnev, 1994).

Because the physical and geochemical properties of water systems depend on the water

[1] Some algae are mixotropic or facultative heterotrophs, some have lost their ability to photosynthesize and become obligate heterotrophs, and some exist in the soil environment.

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flowing into them, they change significantly as a result of urbanization, industrial waste, agriculture, and human activity (Bornette və Puijalon, 2011; Chappuis et al., 2014; Carle et al., 2005). These activities affect the quality and quantity of water, the distribution and diversity of aquatic organisms, primary production and thus, the balance of the aquatic ecosystem (Bornette və Puijalon, 2011).

Depending on the degree of pollution of reservoirs, it is important to study the species that make up the sampling system of reservoirs (Barinova et al., 2019). The following zones differing in the degree of water pollution in the saprobe system are distinguished: catarob (c), xenosaprob (x), oligosaprob (o), α-mesosaprob (α), β-mesosaprob (β), polysaprob (p), isosaprob (i), metasaprob (m), hipersaprob (h), ultrasaprob (u), antisaprobic (a), radiosaprob (r), and kryptosaprob (k) (Barinova and Medvedeva, 1996; Barinova et al., 2006). Catarobic and xenosaprob zones are characterized by a very pure water mass. Oligosaprob zone is considered a completely clean zone of water reservoirs. The water of the oliqosaprob zone is usually saturated with oxygen. In the mesosaprob zone, the degree of water pollution is relatively small, proteins are completely dissolved, and hydrogen sulfide and carbon dioxide are in small quantities.

The physicochemical characteristics of the water system and the socio-ecological characteristics of the water basin also have a significant effect on the species composition of algae in different places along the stream water and lake systems. As the impact of anthropogenic factors on aquatic ecosystems is constantly increasing due to the rapid development of industry, it is necessary to evaluate the species composition and vital activity of algae in rivers, seas, and lakes exposed to anthropogenic influences in order to assess the biological diversity, protection, and sustainable use of algae, the driving forces affecting them and the quality of life of people in a multidisciplinary context.

The Caspian Sea also has a great diversity of algae. It is attributed to its weak connection with other seas and lakes. Besides, it is a closed water basin to some extent. Algae monitoring in the Azerbaijani sector of the sea began in the middle of the 20th century. Previously, in the research conducted in the Caspian Sea, a total of 76 species of algae belonging to 4 divisions, 6 classes, 15 orders, 21 families, and 42 genera were found, of which 38 species and 1 subspecies were found to belong to Baku Bay, and 12 species (8 green, 3 red and 1 black) to the southern shores of the Caspian Sea (Zaberzhinskaya, 1968). As a result of other studies, 309 species and an intraspecific taxon belonging to two classes, three orders, three sub-orders, and eight families of diatoms were recorded in the Caspian Sea (Karayeva, 1972). Multi-year original studies and literature data related to phytoplankton - blue-green algae of the Caspian Sea revealed 85 species and intraspecies taxa, of which only 18 taxa were shown to belong to the Azerbaijani sector of the sea (Zaberzhinskaya, 1968).

In recent years, as a result of the intensification of navigation and the increase in transportation, the discharge of chemical, industrial, agricultural, and domestic wastes, the Caspian Sea, including littoral areas and small water bodies close to these areas, is exposed to pollution, which has a negative impact on water quality, ecosystems and the environment (Shah və Shah, 2013; Niraula, 2012). This pollution may seriously affect the algal biodiversity and bioresources of the aquatic environment. Since monitoring of the Azerbaijani sector of the Caspian Sea has not been carried out for a very long period or has been carried out superficially, it is impossible to obtain sufficiently accurate information on new and rare species of algae, as well as on extinct species.

This article provides information on the taxonomic structure, rare and new species of algae biodiversity identified by monitoring the littoral areas of the Caspian Sea in the Siyazan and Shabran districts of the Republic of Azerbaijan. Ecobiological analysis of the studied species was carried out.

MATERIALS AND METHODS

Algological samples for the study were taken from littoral areas of the Caspian Sea in the Siyazan and Shabran districts located in the northeastern part of the Greater Caucasus. The Siyazan district is located in the north part of Azerbaijan, 103 km north of Baku city in the

Samur-Davachi lowland, on the shore of the Caspian Sea near the Greater Caucasus. It borders Shabran to the west and northwest, the Khizi district to the southeast, and the Caspian Sea to the east. The Shabran district is located in the northeast of the Greater Caucasus, 123 km from Baku, and is surrounded by the Caspian Sea at a distance of 24 km from the east.

In May-September 2023, a total of 40 samples of plankton, benthos, and epiphyte (species attached to the substrate or stones) were collected from 13 sampling points of the Caspian coast of the Siyazan and Shabran districts. The coordinates of the sampling points were determined. Maps showing sampling points are illustrated in Figure 1.

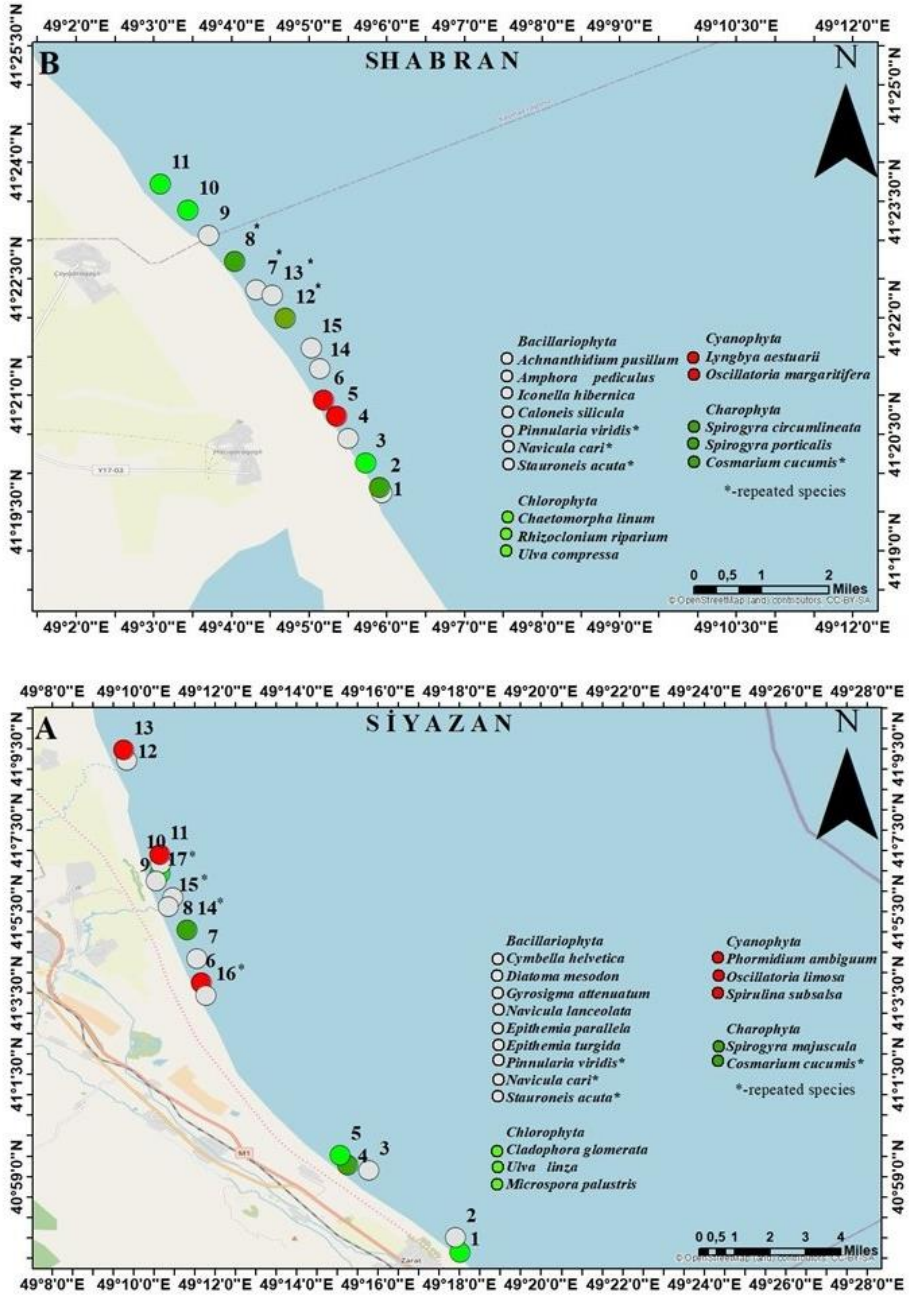

Fig. 1. Algae distribution in littoral areas of the Siyazan and Shabran districts of the Caspian Sea.

Plankton nets (silk gas 25/77) and "Jeddi" nets were used to collect plankton samples. A 3-liter glass container and a bathometer were used for benthic sampling. Epiphytic algae were collected by scraping off the solid substrate with a lancet and transferred to plastic containers (Yashnov, 1934; Arenshteyn, 1961; Boqorov, 1965). To study epilithic diatoms, bottom sediments were sampled 2-3 m from shore using an 8 mm diameter and 1 m long glass tube and transferred to plastic containers. For preservation and detailed study, 10% and 4% formaldehyde was added to epiphytic algae and other ecological group algae, respectively (Tash and Okush, 2006).

The epilithic samples precipitated, the water was drained and the sediment was transferred to Petri dishes. To remove acid and organic matter it was boiled and centrifuged after adding H_2O_2. Permanent samples were prepared from the cleaned materials for diatom identification (Gollerbax and Polyanskiy, 1951; Van Der Werff, 1999). A scanning electron microscope (JSM-35, Japan) was used to study diatoms, and an optical microscope (Nikon E100) was used to identify other algae divisions.

Water temperature was measured with a laboratory thermometer, pH was determined with a portable WTW-3110 pH meter (Germany). The map-scheme of the researched districts was prepared with ArcGIS version 10.7. Literature data were used for morphological characterization and identification of algae (Williams, 1985; Schmid, 1994; Stenina, 2009; Fourtanier and Kociolek, 2011; Seckbach and Kociolek 2011; Kulikovski et al., 2014; Afanasyev et al., 2016; Mukhtarova and Jafarova, 2020; Nuriyeva, 2019; Afanasyev et al., 2020; Volkova et al., 2020).

AlgaeBase (Guiry and Guiry, 2022), “California Academy” (www.calacademy.org), and “Alga Terra” (www.algaterra.org) websites were used to specify the names of algae species, referring to the latest nomenclature.

RESULTS AND DISCUSSION

Taxonomic analysis. As a result of the analysis of algae samples taken from the sampling points along the coast of the Caspian Sea, 28 species belonging to 4 divisions, 14 orders, 16 families, and 21 genera were identified for those areas. These species belong to the following divisions: *Bacillariophyta* (13 species), *Chlorophyta* (6 species), *Cyanophyta* (5 species), and *Charophyta* (4 species). The distribution of the points where algae samples were found along the coast, the coordinates where they were found are shown in Figure 1, Table 1, and Table 3. Microphotographs of algae samples collected using optical and electron microscopes, respectively, from the coasts of Shabran and Siyazan are shown in Figures 2 and 3.

As can be seen in Table 1 and Table 3, *Bacillariophyta* was the dominant group in the algal flora of the district with 46.4%, *Chlorophyta* with 21.4%, *Cyanophyta* with 18%, and *Charophyta* with 14.2% representatives. *Naviculaceae* Kützing, *Oscillatoriaceae* Engler, *Spirogyraceae* Bessey, *Cladophoraceae* Wille were the leading families in algae flora. 22 out of 28 algae species have been newly registered for this district. As seen in the table, algae can be found in various places on the sea coast - littoral areas, estuaries, rocks, in shells and plants (epiphytic), etc. Monitoring of the dynamics of the spread of detected algae by month showed that their spread was relatively higher in May compared to other months. This is likely due to the process of algal blooms that begin in the warmer months depending on environmental conditions.

Ecological analysis. The results of our research showed that 16 species of the detected algal samples are benthic for the algoflora of the Caspian Sea, 7 species are both planktonic and benthic, and 4 species are planktonic (Tables 2 and 3).

Depending on the degree of pollution, water basins are characterized by zones included in the saprob system (Barinova et al., 2019), and the detected algae species were analyzed according to this system. The species were evaluated based on the degree of water pollution in the saprobic system and one species of β-mesosaprobe, one species of xeno-β-mesosaprobe, one species of oligo-α-mesosaprobe, one species of oligo-β-saprobe, one species of xeno-saprobe, two species of oligo-β - mesosaprobe, two species of α-β-mesosaprobe, two species of oligo-xenosaprobe, and two species of oligosaprobe were identified. Such differences may be related to the changes in the geochemical properties of coastal waters and the properties of the water discharged into them.

Table 1. Algae sampling points on the shores of the Siyazan and Shabran districts of the Caspian Sea

Division	Species	Sampling points	Habitat
Bacillariophyta	*Cymbella helvetica* Kützing	40°57.506'N; 49°17.901'E	Estuaries
	Diatoma mesodon (Ehrenberg) Kützing	40°59.156'N; 49°15.775'E	Estuaries
	Gyrosigma attenuatum (Kützing) Rabenhorst	41°4.347'N; 49°11.558'E	Surfaces of mollusc shells
	Navicula lanceolata (C. Agardh) Ehrenb	41°5.850'N; 49°10.960'E	Estuaries
	Epithemia parallela (Grunow) Ruck & Nakov	41°6.687'N; 49°10.674'E	Sea foam
	Epithemia turgida (Ehrenberg) Kützing	41°9.228'N; 49°9.836'E	Sand
	Achnanthidium pusillum (Grunow) Czarnecki	41°19.346'N; 49°6.200'E	Littoral
	Amphora pediculus (Kützing) Grunow	41°20.453'N; 49°5.505'E	Macrophytic algae surface
	Iconella hibernica (Ehrenberg) Ruck & Nakov	41°22.365'N; 49°4.327'E	Littoral area
	Caloneis silicula (Ehrenberg) Cleve	41°23.071'N; 49°3.712'E	Littoral
Chlorophyta	*Chaetomorpha linum* (O.F.Müller) Kützing	41°20.136'N; 49°5.730'E	Littoral and upper sublittoral areas
	Cladophora glomerata (Linnaeus) Kützing	40°57.146'N; 49°18.007'E	Shell surfaces
	Microspora palustris Wichmann	41°6.451'N; 49°10.650'E	Sea foam
	Rhizoclonium riparium (Roth) Harvey	41°23.391'N; 49°3.446'E	On plants
	Ulva linza Linnaeus	40°59.530'N; 49°15.069'E	In the sublittoral zone, on stones and rocks
	Ulva compressa Linnaeus	41°23.732'N; 49°3.089'E	On the substrate, littoral
Cyanophyta	*Phormidium ambiguum* Gomont	41°3.765'N; 49°11.673'E	Littoral
	Oscillatoria limosa Agardh ex Gomont	41°6.910'N; 49°10.640'E	Rocks in littoral and upper sublittoral areas
	Spirulina subsalsa Oersted ex Gomont	41°9.470'N; 49°9.758'E	Shell surfaces
	Lyngbya aestuarii Liebman ex Gomont	41°20.737'N; 49°5.362'E	Littoral
	Oscillatoria margaritifera Kütz ex Gomont	41°20.947'N; 49°5.189'E	Rocks in littoral and upper sublittoral areas
Charophyta	*Spirogyra majuscula* Kützing	40°59.291'N; 49°15.252'E	The surface of macrophytic algae
	Spirogyra circumlineata Transeau	41°19.818'N; 49°5.913'E	Estuaries
	Spirogyra porticalis (O.F.Müller) Dumortier	41°22.739'N; 49°4.042'E	Sea foam

Thus, several small rivers (Devachichay, Shabranchay, Valvalechay, and Atachay), industrial and domestic waters flow into the research areas. Nevertheless, as seen in Tables 2 and 3, the waters of the studied area can be considered weakly or moderately polluted with organic matter and waste.

The littoral zone makes up 7% of the world's oceans, and seas, depending on the ecological characteristics, the structure of the organism, and the relationship between the organism and the environment. The main reasons for the abundance of fauna and flora in this zone are the rich vegetation consisting of algae from the shallow coasts entering the littoral and sufficient food products brought to the coastal zone by the continent waters. The second main reason for species richness is the diverse biotope of the littoral zone (gravelly, sandy, and clayey soils, fresh water at river sources, and dense forests with various plants). Based on the distribution of nutrients and environmental factors, the littoral zone is divided into 3 sub-zones (upper-, supra-, and sublittoral) that differ in terms of ecological conditions (Brauns et al., 2008). A comparative analysis of algae collection areas showed that 13 species of the discovered and studied ones can be attributed to the upper-, 9 species to the supra-, and 6 species to the sublittoral zone. The distribution of those species by subzones is shown in Tables 2 and 3.

Despite the closeness of their areas, the study of the materials collected from the coasts of Siyazan and Shabran revealed only 4 algae species – *Cosmarium curcumis, Pinnularia viridis, Navicula cari,* and *Stauroneis acuta* – in both districts. The characteristics of these species are shown in Table 3, and their images taken under the light and electron microscope are shown in Fig. 4. As seen in the table, these species were mainly benthic, thus, *Cosmarium curcumis* occurred in upper-, *Navicula cari* in supra-, *Pinnularia viridis* and *Stauroneis acuta* in sublittoral zones. It should also be noted that the *Pinnularia viridis* and *Stauroneis acuta* species have an oligo-xenosaprobic saprobic index.

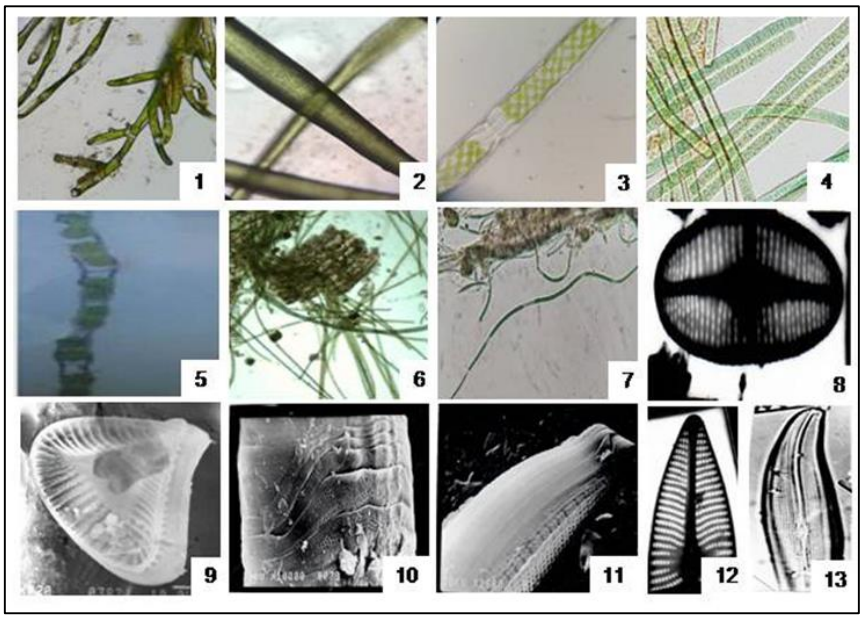

Fig. 2. View of species under the scanning electron microscope and light microscope (Siyazan district): 1. *Cladophora glomerata* (Linnaeus) Kützing, 2. *Ulva linza* Linnaeus, 3. *Spirogyra majuscula* Kützing, 4. *Phormidium ambiguum* Gomont, 5. *Microspora palustris* Wichmann, 6. *Oscillatoria limosa* Agardh ex Gomont, 7. *Spirulina subsalsa* Oersted ex Gomont, 8. *Cymbella helvetica* Kützing, 9. *Diatoma mesodon* (Ehrenberg) Kützing, 10. *Gyrosigma attenuatum* (Kützing) Rabenhorst, 11. *Navicula lanceolata* (C.Agardh) Ehrenb, 12. *Epithemia parallela* (Grunow) Ruck & Nakov, 13. *Epithemia turgida* (Ehrenberg) Kützing.

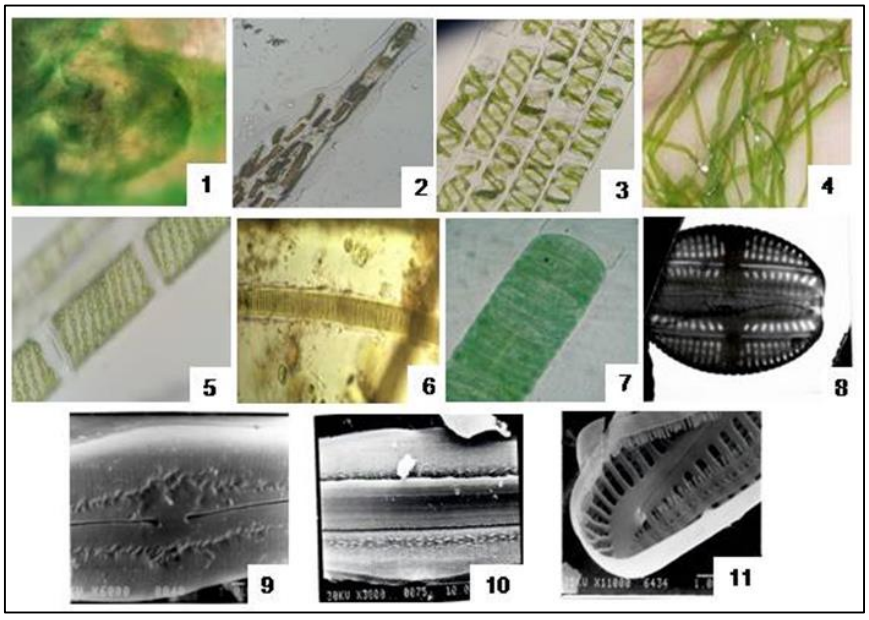

Fig. 3. View of species under the scanning electron microscope and light microscope (Shabran district): 1. *Chaetomorpha linum* (O.F.Müller) Kützing, 2. *Rhizoclonium riparium* (Roth) Harvey, 3. Spirogyra particalis (O.F.Müller), 4. *Ulva compressa* Linnaeus, 5. *Spirogyra circumlineata* Transeau, 6. *Lyngbya aestuarii* Liebman ex Gomont, 7. *Oscillatoria margaritifera* Kütz ex Gomont 8. *Achnanthidium pusillum* (Grunow) Czarnecki, 9. *Amphora pediculus* (Kützing) Grunow, 10. *Iconella hibernica* (Ehrenberg) Ruck & Nakov, 11. *Caloneis silicula* (Ehrenberg) Cleve.

Table 2. Ecological indicators of algae found on the shores of the Siyazan and Shabran districts of the Caspian Sea

Species	Saprob	Ecol. group	Ecological zone		
			Littoral zone (intertial)		
			Upperlittoral	Supra-	Sub-
Cymbella helvetica Kützing	–	ben.		+	
Diatoma mesodon (Ehrenberg) Kützing	o-β	ben.	+		
Gyrosigma attenuatum (Kützing) Rabenhorst	–	pl.		+	
Navicula lanceolata (C. Agardh) Ehrenb	x- β	ben.	+		
Epithemia parallela (Grunow) Ruck & Nakov	–	ben.		+	
Epithemia turgida (Ehrenberg) Kützing	o	ben.	+		
Achnanthidium pusillum (Grunow) Czarnecki	–	ben.	+		
Amphora pediculus (Kützing) Grunow	–	ben.		+	
Iconella hibernica (Ehrenberg) Ruck & Nakov	–	ben.	+		
Caloneis silicula (Ehrenberg) Cleve	x	ben.	+		
Chaetomorpha linum (O.F.Müller) Kützing	–	pl., ben.			+
Cladophora glomerata (Linnaeus) Kützing	β-o	pl., ben.	+		
Microspora palustris Wichmann		ben.		+	
Rhizoclonium riparium (Roth) Harvey	–	pl.		+	
Ulva linza Linnaeus	–	pl., ben.			+
Ulva compressa Linnaeus	–	pl., ben.	+		
Phormidium ambiguum Gomont		ben.	+		
Oscillatoria limosa Agardh ex Gomont	β	pl., ben.			+
Spirulina subsalsa Oersted ex Gomont	o-β	pl., ben.	+		
Lyngbya aestuarii Liebman ex Gomont	o	pl., ben.	+		
Oscillatoria margaritifera Kütz ex Gomont	–	pl.			+
Spirogyra majuscula Kützing	o-α	pl.		+	
Spirogyra circumlineata Transeau	α-β	ben.	+		
Spirogyra porticalis (O.F.Müller) Dumortier	α-β	ben.		+	

Note: pl. – plankton, ben. – benthos, β – betamezosaprob, x-β – kseno-betasaprob, o – oliqosaprob, α-β – alfabetamezosaprob, o-α – oliqoalfamezosaprob, o-β – oliqobetamezosaprob, β-o – oliqobetasaprob, x – ksenosaprob.

Table 3. Characteristics of the same species found in the Siyazan and Shabran districts

Division	Species	Sampling point	Habitat	Saprob	Ecol. group	Ecological zone		
						Littoral zona (intertial)		
						Upper-	Supra-	Sub-
Charophyt	*Cosmarium curcumis* Corda ex Ralfs	41º5.065´N* 49º11.304´E	Sea foam	–	ben.	+		
		41º22.005´N** 49º4.695´E						
Bacillariophyta	*Pinnularia viridis* (Nitzch) Ehrenberg	41º5.625´N* 49º10.849´E	At different depths of water	o-x	ben.			+
		41º22.295´N** 49º4.534´E						
	Navicula cari Ehrenberg	41º3.446´N* 49º11.771´E	Rock surfaces	–	eph. ben.		+	
		41º21.353´N** 49º5.138´E						
	Stauroneis acuta W.Smith	41º6.262´N* 49º10.562´E	At different depths of water	o-x	ben.			+
		41º21.545´N* 49º4.987´E**						

Note: * – Siyazan district, ** – Shabran district, eph. – ephilitic, ben. – benthos, o-x – oligo-xenosaprobic.

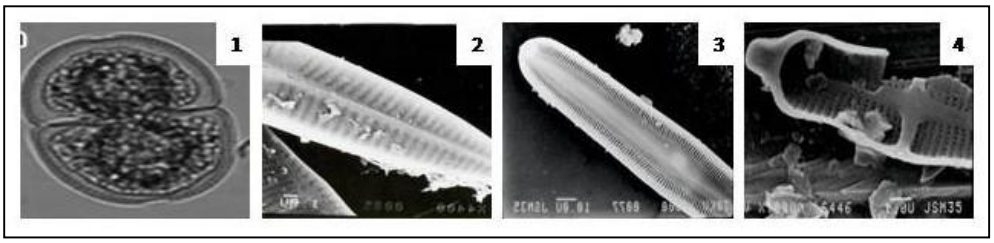

Fig. 4. View of species under the light microscope and scanning electron microscope (Siyazan, Shabran): 1. *Cosmarium cucumis* Corda ex Ralfs, 2. *Pinnularia viridis* (Nitzsch) Ehrenberg, *3*. *Navicula cari* Ehrenberg, 4. *Stauroneis acuta* W. Smith.

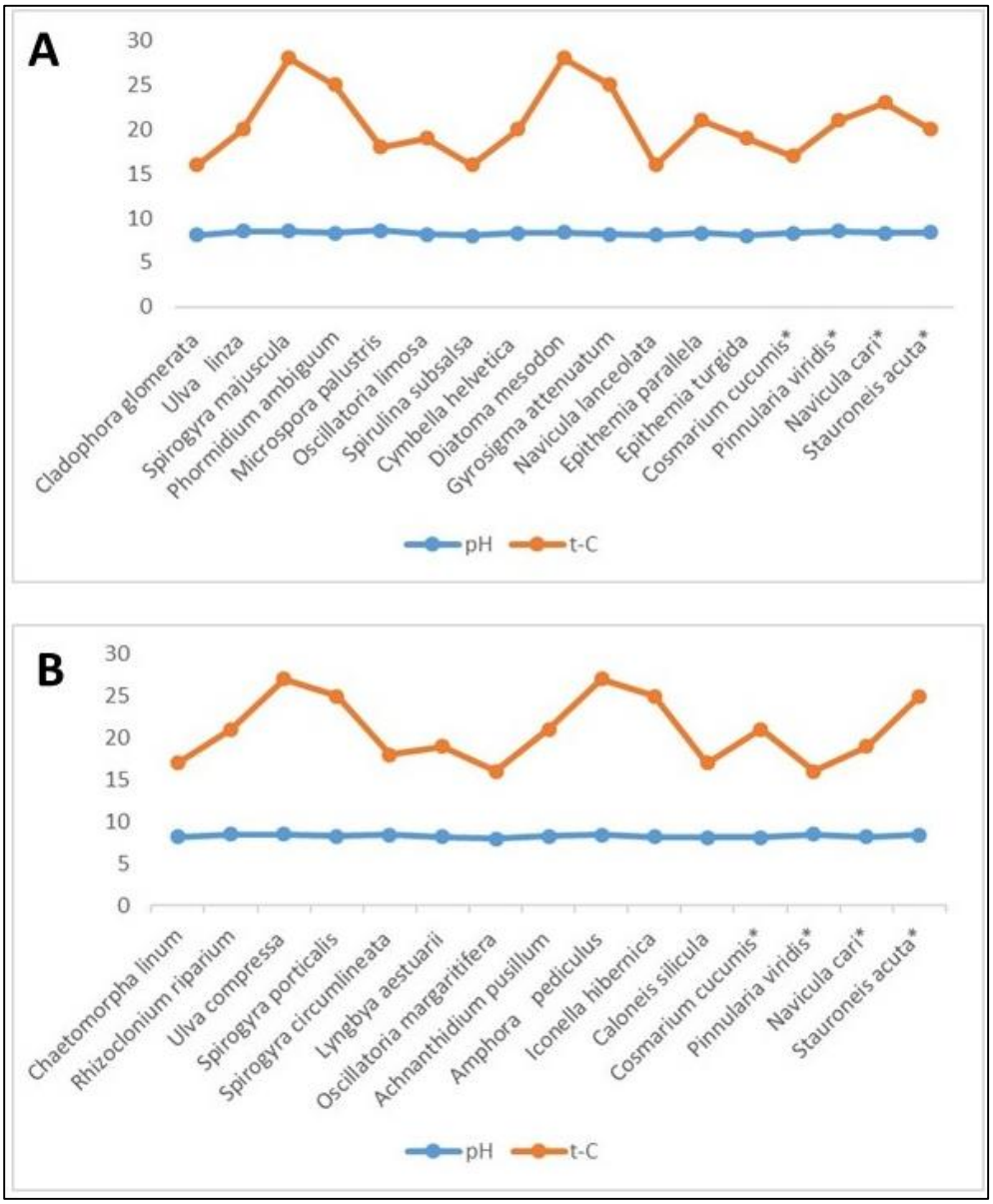

Fig. 5. Ecological conditions of the area where algae spread on the Siyazan (A) and Shabran (B) shores of the Caspian Sea.

Since algae samples were collected in different seasons, the temperature of the sea water in the areas where the species were collected (Figure 1) varied between 16 and 28°C. As seen in Figure 5, some species (*S. majuscula*, *N. lanceolata*, *E. parallela*, *A pediculus*, and *C. glomerata*) were detected at relatively low (16-20°C) temperatures, two of which – *C. cucumis* and *P. viridis* are common algal species. The average pH value of sea water in the areas where the experiments were conducted was 8.0 with slight differences. This indicator is close to the general pH indicator of the Caspian Sea (8.3). Thus, unlike other seas and oceans, the pH in the waters of the Caspian Sea is relatively high. The pH in the upper layers of seawater varies between 8.2 and 8.6, and in the deep layers between 7.9 and 8.1. Alkalinity in the Caspian Sea varies both seasonally and geographically, decreasing with increasing depth. It remains stable only in the open areas of the sea.

CONCLUSION

The Caspian Sea has a unique and rich fauna and flora. The uniqueness and variety of natural conditions, the weak connection with the sea and oceans have ensured the existence of many rare species of fauna and flora in the Caspian Sea. In the Azerbaijani sector, the water of two large rivers (Kur and Araz) and many small rivers, including industrial waste, are poured into the Caspian Sea. Both rivers pass through the territory of bordering countries, many polluted small rivers flow into them (Okhchuchay, Basitchay), and eventually enter the Azerbaijani sector of the Caspian Sea. This, in turn, is one of the factors that seriously affect the algae spectrum of the sea. Algae growth and reproduction are also affected by light, temperature, inorganic substances, and other factors. The fact that the research district is located outside the Kura River estuary and industrial regions and the relatively clean water affects the algal biodiversity of the area is also of interest. The discovery of new species that have not been recorded for this small area shows that the whole Caspian algoflora has been enriched in the last 50 years including the Azerbaijani sector. The study of the role of various abiotic factors in the formation of individual regions of the Caspian Sea, as well as the algoflora of the Caspian water area as a whole, has become one of the important regional issues and requires extensive research.

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CONFLICT OF INTEREST

There is no conflict of interest in the present study.

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Comparative analysis of the activity of AZ-130 and *B. subtilis* supernatants against *Lactococcus lactis*

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The aim of the presented work was to compare the activities of supernatants collected from *Bacillus vallismortis* strain AZ-130 and *B. subtilis* strains against *Lactococcus lactis* subsp. Lactis ATCC 11454. To achieve the goals, cultures of AZ-130 and *B. subtilis* and their SN were analyzed for activity against *Lactococcus lactis* subsp. Lactis ATCC 11454 by broth microdilution and growth inhibition assay. Based on the obtained results, it was found that strains AZ-130 and *B. subtilis* have high activity in the supernatant against *Lactococcus lactis* subsp. Lactis ATCC 11454 strain. However, given that the inoculation and growth conditions of both strains were the same, the activity of AZ-130 is significantly (16-fold) higher compared to the supernatant of *B. subtilis*. The presence of activity in the supernatants of AZ-130 and *B. subtilis* against *L. lactis* indicates a possible similarity of the antimicrobial compound produced by strain AZ-130 with the antimicrobial compounds produced by *B. subtilis.*

***Keywords:** Antimicrobial activity, bioactive molecules, natural products, pathogenic bacteria*

INTRODUCTION

The gradual acquisition of resistance by microorganisms to clinically used antimicrobial drugs represents a serious health problem and requires the development of new antimicrobial drugs (Armas et al., 2019; Zaman et al., 2017). Discovery and research of natural products that are produced by various organisms (plants, terrestrial vertebrates and invertebrates, marine organisms, bacteria and fungi) (Abdel-Razek et al., 2020), have provided many active and leading structures for pharmaceutical development (Schneider, 2021; Atanasov et al., 2021). Natural antimicrobials with widely varying chemical structures and biological activity play an important role in medicine, agriculture, and also in the food industry from the point of view of food safety from foodborne pathogens (Pham et al., 2019). According to Newman and Cragg 70% of antibacterial drugs on the market from 1981 to 2019 are natural products or their derivatives, 28% - synthetic drugs, 1% - imitation of natural products and pharmacophores (Schneider, 2021). Microorganisms are the most potential source for the production of natural antibacterial drugs (Wright, 2014). Isolation, purification and identification of natural antimicrobial bioactive compounds is a very time-consuming and financially demanding process (Ekins et al., 2019; Wright, 2018). However, in most cases, at later stages of development, it turns out that the molecule under study has been previously identified. One of the main steps in antimicrobial development from natural sources is to include dereplication stages in the process to avoid the re-development of already known compounds (Schneider, 2021; Carrano and Marinelli, 2015).

An AZ-130 strain, isolated from oil contaminated soil sample of Azerbaijan, showed strong activity against gram-positive opportunistic pathogenic *S. aureus* and *E. faecalis* strains

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(Araeва, 2019; Aghayeva et al., 2021) during initial and supernatant screenings. By 16S rRNA gene sequencing AZ-130 strain was identified as *Bacillus vallismortis*. Further efforts to characterize the AZ-130 bioactive compound showed that strain AZ-130 produces a single compound with antibacterial activity with a retention time at HPLC column 12.854 min (Aghayeva et al., 2021). Bacterium *B. vallismortis,* to which strain AZ-130 belongs, is very similar to *B. subtilis* (Roberts et al., 1996; Earl et al., 2012). The soil microorganism *B. subtilis* stands out among members of the genus *Bacillus* because it produces many different potential antibiotics (Caulier et al., 2019; Stein, 2005). In addition, *B. subtilis* produces a number of peptide antibiotics, including members of both classes: ribosomal synthesized (for example, subtilin, subtilosin A (Shelburne et al., 2007) ericin A and S, mersacidin, sublancin 168, bacillocin 22) (Lawton et al, 2007; Xie et al., 2009) and several types of non-ribosomally synthesized small antibiotic peptides (<2000 Da) that exhibit antibacterial and antifungal activity (for example, iturin) and lipopeptides such as surfactin, fengycin, mycosubtilin, and mycobacillin (Li et al., 2009).

The molecular weight of the AZ-130 compound is more than 3000 Da (Aghayeva et al., 2022). One of the antimicrobial compounds produced by *B. subtilis* bacterium with a molecular weight above 3000 Dalton is subtilin (3321 Da) (Subtilin). It is known, that subtilin, produced by *B. subtilis* inhibits the growth and development of *L. lactis* (Qin et al., 2019; Parisot et al., 2008). Since these two strains are closely relative to each other and may produce similar antimicrobial compounds, the purpose of the experiments presented in this work was to elucidate the similarities and differences in the mechanisms of activity of the antibacterial compounds produced by AZ-130 and *B. subtilis* strains against *Lactococcus lactis* subsp. Lactis ATCC 11454.

MATERIALS AND METHODS

The object of study was an AZ-130 antibacterial compound synthesized by the *Bacillus vallismortis* strain AZ-130 isolated from an oil-contaminated soil sample of Azerbaijan in 2014.

The *B. subtilis* strain was obtained from the Fraunhofer Mid-Atlantic Center, USA and identified by 16S rRNA gene sequencing as *Bacillus subtilis ssp. spizizenii str.* NBRC 101239.

50 ml of TB medium was added to two 125 ml flasks: one for AZ-130, the second for *B. subtilis*. Flasks were inoculated with one colony of AZ-130 (or *B. subtilis*) and incubated at 220 rpm and 32°C for 24 hours. After the incubation time, the culture was centrifuged at 10000 g for 15 min at 4° C and the supernatant was purified from the cell culture by filtration through a 0.22 μm PES membrane. Culture and supernatant of strains AZ-130 and *B. subtilis* were assayed for activity against *L. Lactis* by the growth inhibition assay. The screening was performed by the soft-agar overlay method as described by Hockett (Hockett and Baltrus, 2017; Balouiri et al., 2016) with some modifications. For screening, 10 μl of material was plated onto an agar plate confluent with the indicator strain - *Lactococcus lactis* subsp. Lactis ATCC 11454. The plates were left to dry for 5 minutes under a hood and incubated at 37°C for 24 hours. The range of antibacterial activity (zone of inhibition (ZOI)) was expressed in millimeters as the diameter of the transparent zone (the zone where the growth of the test organism was suppressed).

To compare the number of inhibitory units secreted by strains AZ-130 and *B. subtilis* over 24 hours, the collected SNs were diluted and assayed against *L. lactis* by the broth microdilution method (Manual…, 2005) according to recommendations of the Clinical and Laboratory Standards Institute (CLSI). The experiment was repeated three times. The supernatant (100 μL) was added to the first well of a 96-well plate and two-fold serially diluted across the row. Cell suspension of *Lactococcus lactis* subsp. Lactis ATCC 11454 (50 μL) was added to each well to the final concentration of 5×10^4 cells per well. For the positive control (100% growth of the test organism), 50 μL of the medium was mixed with 50 μL of the test organism suspension; pure medium without the test organism (100 μL) was used as the negative control. The plates were covered with a lid and incubated at 37°C for 22-24 hours in an open bag (to prevent moisture loss). After the incubation time, OD was measured at 650 nm using a Molecular Devices Spectra MaxPlus microplate reader.

RESULTS AND DISCUSSION

To determine the similarity/difference in the mechanism of activity of the antimicrobial compounds produced by strains AZ-130 and *B. subtilis*, SNs of AZ-130 and *B. subtilis* were analyzed for activity against *L. lactis*.

As can be seen from Figure 1, AZ-130 and *B. subtilis* show very faint activity in culture against *L. lactis*, while the SN activity of the same isolates was quite high: 8 mm – SN of strain AZ-130 and 5 mm – SN of strain *B. subtilis* (Fig. 1).

Based on the results of the growth inhibition assay, it is clear that the activity of the AZ-130 supernatant against *L. lactis* is higher compared to *B. subtilis*. It should be noted that the inoculation and growth conditions for AZ-130 and *B. subtilis* were the same. To be able to compare the number of inhibitory units secreted by these strains over 24 hours, the collected SNs were diluted and assayed against *L. lactis* by the broth microdilution method (Fig. 2).

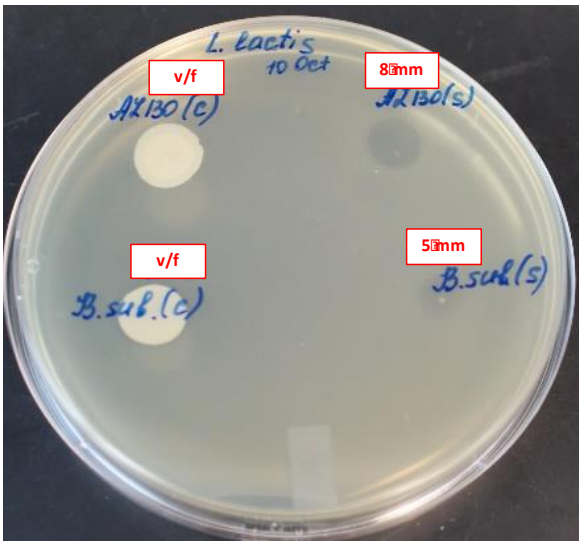

Fig. 1. Antibacterial activity of AZ-130 and *B. subtilis* cultures and their supernatants against *L. lactis*. ***Note:*** *v/f - very faint activity.*

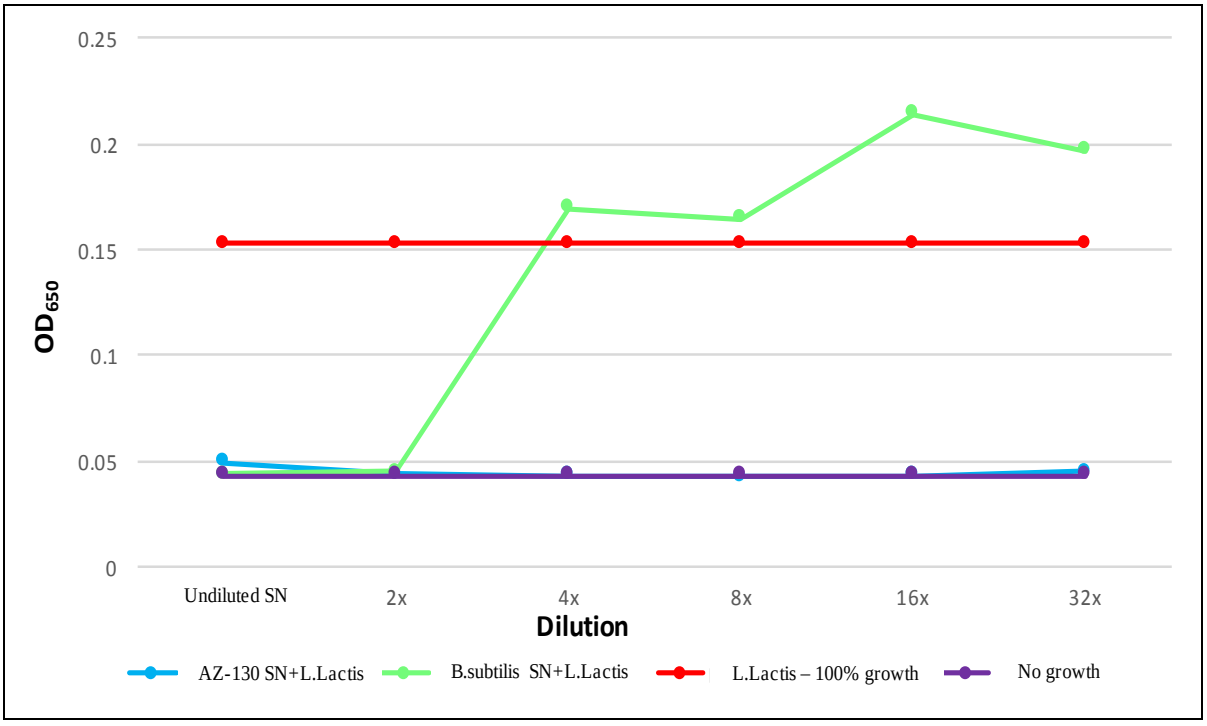

Fig. 2. Antibacterial activity of AZ-130 and *B. subtilis* cultures and their supernatants against *L. lactis*. *Note: v/f - very faint activity.*

In the figure, the maximum growth of *L. lactis* under the tested conditions (no inhibitor) is highlighted in red, the negative control (no bacterium) is in purple, the activity of the AZ-130 supernatant at various dilutions against *L. lactis* is in blue, and the activity of the *B. subtilis* supernatant at various dilutions against *L. lactis* – green. The experiment was carried out three times.

Broth microdilution analysis of the SNs (Fig. 2) showed that the AZ-130 supernatant completely inhibited the growth of the *L. lactis* strain at a 32-fold dilution, whereas the *B. subtilis* supernatant showed activity only at a 2-fold dilution. Analysis of the collected SNs by the broth microdilution method carried out to compare the number of inhibitory units, showed that the activity of the SN of strain AZ-130 against *L. lactis* was 16 times higher than the *B. subtilis* SN.

CONCLUSION

It has been found that the supernatant of strain AZ-130 has a high activity against the bacterium *Lactobacillus lactis*. The similarity in molecular mass of the AZ-130 biomolecule with subtilin produced by *Bacillus subtilis* (more than 3000 Da) and the presence of activity against *L. lactis* indicate the possibility that this antimicrobial compound belongs to the same class as subtilin, but has a 16-fold higher activity compared to subtilin.

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CONFLICT OF INTEREST

There is no conflict of interest in the present study.

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Geobotanical zoning of Karabakh and East Zangezur

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The article discusses the features and characteristics of vegetation Karabakh and East Zangezur region. There are 6 geobotanical areas and 6 geobotanical districts. Taking into account the types of vegetation, a brief geobotanical description of the regions and geobotanical districts is carried out. The region is characterized by desert, semi-desert, wetland, steppe, meadow, shrub, forest and rock-talus vegetation types. This diversity is associated with vertical zonation, which originates from the lowland and rises to the nival belt. The main representatives of the flora of the region are also indicated.

***Keywords:** Geobotanical zoning, vegetation, plant species, height above sea level*

In connection with the liberation of Karabakh and East Zangezur region from 30 years of occupation, these and the territories in contact with it are of particular interest on issues related to natural resources and, in particular, floristic and plant diversity (Ibadullayeva, Huseynova, 2021). In this sense, it becomes important to systematize the vegetation cover of the region, which at this stage, for known reasons, is possible through a cartographic inventory (Hajiyev 2007; Prilipko, 1963; Khalilov et al., 2014), literary (Prilipko, 1973) material and limited field research. In Azerbaijan, the main principles of systematization of vegetation cover include floristic, botanical-geographical and geobotanical, zoning systems. These zoning systems have the goal of establishing the geographical features of flora and vegetation cover. V.B.Sochava (1966), E.M.Gurbanov (2021) characterizing the features of geobotanical and botanical-geographical zoning systems, indicates that these systems have the goal of classifying the territory by vegetation cover. At the same time, the territorial structure of vegetation cover comes to the fore, reflecting the relationship of vegetation with environmental factors, and in regions with vertical zoning, zoning is characterized by special specificity and "…. obeys its own laws." In this work, as the initial stage of inventorying the vegetation cover of Karabakh, we consider the geobotanical zoning of its territory. In our understanding, geobotanical zoning is a generalization of material on the vegetation of a certain territory, taking into account the totality of characteristic or zonal plant communities common in this territory. This takes into account phytocoenotic (combination of zonal and intrazonal types of vegetation, type of geographical landscape, vertical zonality) and floristic characteristics (dominant composition of zonal vegetation or groups of species with the same general distribution associated with a certain territory). In the hierarchical ladder of division, we adopted geobotanical areas and geobotanical districts. By the term "geobotanical area" we mean the leading zonal landscape type of vegetation characteristic of the region, taking into account the height above sea level. In the name of formations, we have chosen ecological-phytocoenotic from existing vegetation classification systems (Neshataev, 2001; Movsumova, 2005; Gurbanov, 2007), since until now, the systematization of vegetation by Caucasian researchers, including

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Azerbaijani ones, has been carried out following this principle. In addition, this approach facilitates the comparative analysis of current plant communities with communities of previous years. By the term "geobotanical district" we mean an administrative-geographical unit in which a given zonal type of vegetation is observed. Plant names are given in accordance with "Flora of Azerbaijan" (1950-1961) and "The World Flora Online".

Since the distribution of vegetation in the territory is closely related to physical and geographical conditions, below we provide a description of the main natural indicators of Karabakh and East Zangezur region (Khalilov et al., 2014). Karabakh belongs to the southeastern part of the Lesser Caucasus. According to the botanical and geographical division of Azerbaijan, it includes 2 areas - Lesser Caucasus Central (LC central) and the Lesser Caucasus South (LC South). The relief is mostly mountainous. The highest point is Mount Gamish (3724 m), the others are Mount Gizgala (2843 m), Mount Kirkhgiz (2827 m), Mount Big Kirs (2725 m). The Murovdagh and Karabakh ridges are divided into several side branches descending to the Karabakh and Mil plains. The geological structure of the secondary tectonic elements of the Lesser Caucasus is occupied by the Murovdagh, Karabakh, Aghdam anticlinoriums and the rivers Toragaychay and Khojavend synclinoriums separating them from each other. The river network is formed by the main River Araz. The high mountain peaks located here form a watershed between the basins of the Rivers Araz, Tartar, Arpachay, and Bazarchay. The Rivers Tartarchay, Khachinchay, Gargarchay, Hakarichay, Okchuchay, flowing along the southeastern slope of the Lesser Caucasus, are of great importance in irrigating the Mil and Karabakh plains. The river network crossing the territory created deep and steep valleys. In terms of climate, in the highlands, the annual precipitation exceeds 800-900 mm. The high peaks of the Murovdagh ridge are covered with snow all year round. The river network of the territory is represented by the Kura River with tributaries of the Rivers Tartar, Khachin, Gargar and Araz, Ondalanchay, Guruchay, Gozluchay, etc. Soils vary from chestnut, light chestnut, mountain-dark chestnut, brown mountain forest, mountain meadow to lowland grey-brown, brown saline. Flora and vegetation are subject to vertical zoning (Gurbanov, 2018).

In Karabakh and East Zangezur, there are 6 geobotanical regions and 6 geobotanical districts (Table 1).

Table 1. Classification of geobotanical areas of Karabakh and East Zangezur region

Area	Geobotanical area	Zonal vegetation types
Lesser Caucasus Plain	South Karabakh	Desert, semi-desert, dry steppe
Lesser Caucasuslowlandforest	Barda	Lowland forest, shrubby, lowland meadow, wetland
Lesser Caucasus foothill lower-mid-mountain xerophytic, shrub-forest	Aghoghlan	Arid woodlands, steppe, shrub
Lesser Caucasus lower-mid mountain forest	Lesser Caucasus	Forest, shrubby
Lesser Caucasus high mountain forest, meadow, steppe	Hinaldagh-Dalidagh	Forest, meadow, steppe
Lesser Caucasus nival		Meadow

Geobotanical division and brief description of vegetation cover of Karabakh and East Zangezur region (Figure):

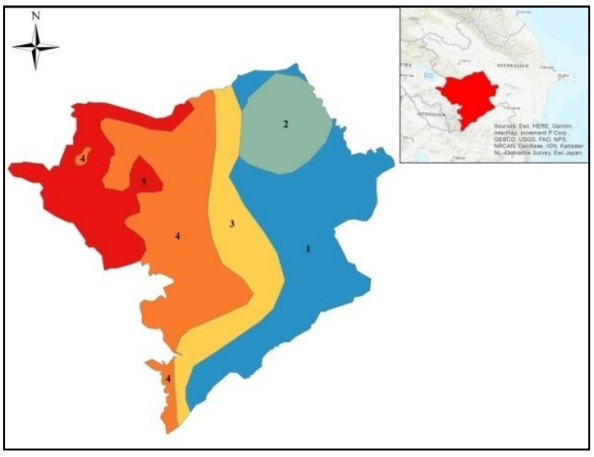

Fig. Distribution of geobotanical areas of Karabakh and East Zangezur region:
1 - South Karabakh; 2 - Barda; 3 - Aghoghlan; 4 - Lesser Caucasus; 5 - Hinaldagh-Dalidagh.

1. *Lesser Caucasus plain area.* The largest area of the region, covering almost 1/3 of the territory. It includes the South–Karabakh geobotanical area, which includes the Aghdam, Aghjabadi, Beylagan, as well as the Tartar, Khojavend, Fuzuli, Jabrayil, Zangilan administrative districts. This part of Karabakh is characterized by lowland, flat terrain. In this regard, the term "aran" was introduced into its name, i.e. lowland, flat. Most part of this area is in contact with the neighboring Kura-Araz lowland, which belongs to the Kura-Araz geobotanical area of the same name. The Upper Karabakh water channel and the Mil Karabakh collector pass through the territory of the area, the operation of which at one time led to the secondary salinization of nearby territories. The area is zonally represented by saltwort desert and semi-desert shrub, subshrub, semi-shrub, semi-subshrub vegetation with dominance of *Salsola dendroides* Pall., *S. ericoides* M.Bieb., *S. nodulosa* (Moq.) Iljin, *Kalidium caspicum* (L.) Ung.-Sternb., *Halocnemum strobilaceum* (Pall.) M.Bieb., annual saltwort with the participation of *Petrosimonia brachiata* (Pall.) Bunge, *Salsola crassa* M.Bieb, wormwood with the dominance of *Seridiphidium fragrans* (Willd.) Poljakov (=*Artemisia fragrans* Willd.), as well as wormwood-saltwort (*Artemiseto - Salsoleta*) vegetation with intrazonal lowland meadow-like groups (*Glycyrrchiza glabra* L., *Cynodon dactylon* (L.) Pers., *Spergularia diandra* (Guss.) Heldr.), as well as semi-desert wormwood-forb, wormwood-saltwort and dry-steppe communities (*Bothriochloeta*).

2. *Lesser Caucasus area of lowland forests.* This area is represented by one Barda geobotanical area and is located on the territory of Barda, partly Aghdam, Tartar administrative districts. The area is characterized by broad-leaved light oak (*Querceta iberica*) and oak-elm (*Querceto – Ulmeta*) forests. Alder forests (*Alneta*) predominate in humid lowland areas. It should be noted that over the past decades, the range of lowland forests has decreased, which requires their inventory. In post-forest places, secondary shrub and forb-grass meadow vegetation develops (*Herbeta*). In swampy areas, swampy meadow vegetation (*Phragmiteta)* with the participation of *Stuckenia pectinata* (L.) Börner, *Calamagrostis epigeios* (L.) Roth) is observed. This area borders the South Karabakh area, which in a certain way leaves an imprint on the vegetation of the border areas of the Barda district, i.e. representatives of the semi-desert can be found in ecotone areas.

3. *Lesser Caucasus area of foothill, lower mid-mountain xerophytic shrub and forest vegetation*. This area in Karabakh is represented by the Aghoghlan geobotanical area. Administratively, it belongs to the Shusha, Khankendi, partially Tartar, Jabrayil, Zangilan districts. A narrow strip stretches from the borders of Naftalan, through Khojali, Khankendi and Shusha to Zangilan, ending at the border with Iran. The area is characterized by a predominance of arid vegetation. This includes mountain steppes, steppe meadows, semi-steppes, arid woodlands and communities of mountain xerophytes. In the lower and mid-mountain zones there are communities of broad-leaved forests (*Querceto – Carpineta*) with tree species *Quercus iberica* M. Bieb., *Carpinus betulus* L., *Fagus orientalis* Lipsky, *Fraxinus excelsior* L. Representatives of conifers can also be found here *Pinus sylvestris* ssp. *hamata* (Steven) Fomin (=*Pinus kochiana* Klotzsch ex K. Koch), *Taxus baccata* L., *Juniperus polycarpos* K. Koch., *J. foetidissima* Willd.

4. *Lesser Caucasus area of mountain forests* with Lesser Caucasus geobotanical area. The area includes vast territories of the Lachin and Gubadli districts, as well as the partially adjacent Zangilan and Shusha districts. The Lesser Caucasus geobotanical area is represented by broad-leaved light and shade forests dominated by oak and hornbeam. In areas of damaged forest, deciduous, drought-resistant shrubs and low-growing trees are observed as secondary vegetation. The zonal representative of shiblyak is *Paliurus spina-christi* Mill. The forests of this area are characterized by such tree species as *Q. iberica*, *F.orientalis*, *C.betulus*, *Fraxinus angustifolia* subsp. *oxycarpa* (Willd.) Franco ex Rocha Afonso (=*Fraxinus oxycarpa* Willd.), as well as wild fruit plants – medlar (*Mespilus germanica* L.), rosehip (*Rosa zangezura* Jarosch.), plum (*Prunus divaricata* L.), cherry (*Prunus mahaleb* L., *P. incana* (Pall.) Batsch). Representatives of conifers *P. sylvestris* ssp. *hamata*, *T. baccata*, *Sorbus torminalis* (L.) Griatz, species of juniper, as well as forb-legume-cereal meadow, meadow-steppe

herbaceous vegetation, with periodically changing dominants, secondary vegetation formed at the place of clearings and shrubs can also be found here.

5. *Lesser Caucasus area of high mountain vegetation*. Administratively, it includes the Kalbajar district, where the famous healing spring Istisu is located. In Karabakh, this area includes the Hinaldagh-Dalidagh geobotanical area. This area is characterized by zonal meadow, steppe meadow, meadow-steppe vegetation. Intrazonally there is primitive vegetation of rocks, as well as shrub groups with the participation of *P. spinachristi*, almond (*Prunus fenzliana* Fritsch, and species of hawthorn genus (*C.pentagyna* Waldst. & Kit. ex Willd., *C.orientalis* (Mill.) M. Bieb., blackberry (*Rubussaxatilis*L.) ash berry (*Sorbus caucasica* Zinserl), bird cherry (*Prunus padus* L.), *Rosa tomentosa* Sm. (=*Rosa cuspidata* M. Bieb.), etc. Against the background of zonal cereal-grass meadow subalpine and alpine vegetation with the participation of *Alchemilla caucasica* Buser, *Betonica macrantha* K. Koch, *Carum caucasicum* Boiss., *Pentanema germanicum* (L.) D. Gut. Larr. Santos-Vicente, Andreb, E.Rico & M.M.Mart. Ort., *Heracleum trachyloma* Fisch. & C.A.Mey, *Sibbaldia parviflora* Willd., *Campanula sibirica* sbsp. *hohenackeri* (Fisch. & C.A.Mey) Damboldt, high-mountain steppes and steppe meadows (*Stipa szovitsiana* (Griseb.) Trin., *Festuca ovina* L., *F. varia* Hack) are developed. In the same area, high-mountain subalpine forest groups of orientalis oak (*Quercus macranthera* Fisch. & C.A.Mey), *F. orientalis*, *P. sylvestris* ssp. *hamata*, *Sorbus caucasica* and birch (*Betula pendula* Roth), and maple (*Acer campestre* L.) occur intrazonally. The post-forest slopes are occupied by meadow vegetation (species of the genera *Calamagrostis* Adans., *Poa* L., *Vicia*L., etc.), in some places by upland xerophytes with the participation of representatives of low-growing and evergreen shrubs (garigue), low-growing xeromorphic shrubs and subshrubs (phrigana) with representatives of the main genera *Acantholimon* Boiss., *Astragalus* L., *Onobrychis* Mill., *Thymus* L., *Ziziphora* L., etc.

6. *Lesser Caucasus nival area*. Since the subnival and nival zones are in terms of vertical zonation and climatic conditions and, accordingly, species composition differs from the subalpine and alpine zones, we considered it logical to distinguish the vegetation of these zones into a separate area. In Karabakh, it is represented by the Hinaldagh-Dalidagh geobotanical area. The vegetation of this belt is characterized by the participation of petrophytes (species of the genera *Woodsia* R.Br., *Saxifraga* L., *Ranunculus* L., etc.), as well as the intrazonal participation of some cold-resistant representatives characteristic of the upper boundary of the alpine belt (*Potentilla aegaea* Boiss., *Nepeta somchetica* O. Capeller).

It is necessary to note the role of rock-talus, stony and pebble vegetation. Despite the presence of rocks, in the upper high-mountain and nival zones, this vegetation in Azerbaijan and in particular in Karabakh is also found on the rocks of the middle and upper mountain zones). In his work "Vegetation Cover of the Caucasus" (1948) A.A. Grosseim notes that the composition and nature of rock-talus vegetation can vary depending on the location of the area above sea level, the type of climate against which a given area of rock-talus vegetation develops, the forms of rock weathering, and the chemical composition of the rock. The first two provisions confirm our opinion about the intrazonality of both this and rocky and pebble vegetation.

As noted above, for objective reasons, there are currently some difficulties in fully studying the vegetation cover of the region. This especially applies to forest vegetation, which has been subjected to the most severe impact. Despite this, in the future, in specific locations of the described part of the region, a complete geobotanical revision of vegetation will be carried out. Despite this, researchers of the Institute of Botany, MSE RA began re-inventorying the flora of the region. In 2023, monitoring was carried out on plants distributed in the Fuzuli, Jabrayil, Zangilan, and Gubadli territories of Azerbaijan liberated from occupation and in many villages included in those territories. As a result of monitoring, the species such as *C. orientalis, Datura stramonium* L., *Cuscuta campestris* Yunck., *Veronica anagallis-aquatica* L., *Lythrum salicaria*L., *Capparis spinosa* L., *Cirsium arvense* (L.) Scop., *Epilobium hirsutum* L. ,*Convolvulus arvensis* L., *Achillea micrantha* Willd., *Robinia pseudoacacia* L., *Populus alba* L.,*Celtis caucasica* Willd., *Punica granatum* L., *Platanus orientalis* L., *Fraxinus excelsior* L., *Salix alba* L., *Ficus carica*

L., *Gleditsia triacanthos* L., *Tribulus terrestris* L., *Ailanthus altissima* (Mill.) Swingle, *P. spina-christi*are still found in liberated lands. Rare and endangered species such as *P.orientalis*, *P.granatum*, *J. foetidissima*, *Ficus carica* L. and many species are sparsely distributed in forest clearings and meadows. The species *Bidenstripartita* L. included in the IUCN Red List was also found in the Guydzhak village of the Jabrayil district.

CONCLUSIONS

Currently, throughout Azerbaijan, changes in natural conditions and the negative impact of human activity (development of agriculture, industry, urbanization, recreation, grazing, organization of recreation areas, cutting down trees) lead to changes in the boundaries of plant landscapes, the emergence of rare plant communities, an increased number of alien plants, the disappearance and reduction of a large number of plant species, the redistribution of the dominant species composition of many plant communities, accordingly, a change in the structure of the latter. In this regard, local botanists plan to inventory and systematize vegetation and flora using new scientific methodological approaches.

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Study of some morphophysiological traits and productivity of winter bread wheat that can be applied in selection under rainfed conditions without moisture supply

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Plant height, flag leaf area, relative water content, stay green period, photosynthesis rate (P_n), stomatal conductance (g_s), transpiration rate (T_r), and productivity of 12 varieties and 9 lines of winter bread wheat with contrasting morphophysiological traits have been studied under various water supply. It was shown that the improvement of water supply in the late period had less effect on plant height, and more effect on flag leaf area, relative water content and stay green period. The relative water content of the flag leaf was 72.9% and 81.1% in drought-exposed and irrigated variants for all genotypes. The relative water content of the flag leaf was higher in Aran, Vostorg, Murov 2, Zirva 85, Tale 38, and Gyrmyzy gul 1 varieties than in other studied genotypes under both drought stress and normal water supply conditions. The study of the stay green period of the flag leaf showed that its average value for all genotypes was 484 GDD under drought stress and 575 GDD under normal water supply conditions. The value of this parameter in Murov 2 and Zirva 85 varieties was higher compared to the other studied genotypes. The highest values of P_n were observed in the varieties Tale 38, Aran, and Gyrmyzy gul 1 in both drought-exposed and irrigated variants. The average productivity of all genotypes under drought was 496 g/m^2, and in the irrigated variants, it amounted to 623 g/m^2. Under drought, the highest productivity was detected in the 7th WON-SA № 465 (607 g/m^2), Gobustan (557 g/m^2), and Ferrigineum 2/19 (549 g/m^2) genotypes. In the irrigated variant, the highest productivity was 728, 717, 707, and 706 g/m^2 in the 7thWON-SA №465, Tale 38, Gyrmyzy gul 1, and Gobustan genotypes, respectively. The results of the study have shown that the potential of the Gyrmyzy gul 1 and Tale 38 varieties is high, and the use of Murov 2 and Zirva 85, and Gyrmyzy gul 1 varieties in hybridization is appropriate.

Keywords: *rainfed conditions, bread wheat, morphophysiological traits, productivity*

INTRODUCTION

Wheat is a plant that occupies an important place in the food supply of people in the world. According to the Food and Agriculture Organization of the United Nations (FAO), wheat is planted on 220-225 million hectares of land in the world and an average of 750 million tons of crops are produced every year (www.fao.org/worldfoodsituation/csdb/en/). In the agricultural production of our republic, wheat occupies the first place in terms of both cultivated area and production. A part of the wheat consumed in our republic is still imported from abroad (worldagriculturalproduction.com/crops/wheat.aspx). Due to the natural climatic conditions, winter cereals are planted most in our country and they are cultivated either with rainfed farming systems or under irrigation conditions where irrigation is possible. It is known that the production process

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can be carried out by applying the cultivation technologies of rainfed farming in places where the amount of total annual rainfall is 250-500 mm. Under such conditions, it is necessary to plant drought-tolerant varieties, whose low productivity leads to a decrease in total grain production. In this regard, the main goal of wheat breeders is to create genotypes with high and stable productivity under drought-stress conditions (Aliyev, 2000, 2001). Under the conditions of drought stress, many changes occur in molecular, biochemical, and physiological processes in the plant aimed at reducing the effect of stress (Bray, et al., 2000). In order to create genotypes that can produce higher yields under stress conditions, it is important to study the physiological and morphological characteristics that play a role in the tolerance of plants to drought (Aliyev, 2000; Allahverdiyev, 2020). The formation of drought tolerance in plants is a very complex and time-consuming process. Therefore, the study of some physiological and morphological characteristics of the plant under different water supply conditions is of great importance (Allahverdiyev, 2016: Hassan et al., 2019). Under drought conditions of Mountainous Shirvan, which are not stably supplied with moisture, drought usually begins in May, at the 47-49 stage of the vegetation of winter bread wheat according to the BBCH (Biologische Bundesanstalt, Bundessortenamt and Chemical Industry) scale. At the same time, in some years, an average or normal supply of moisture in that period was observed. Therefore, in that period, winter bread wheat should use moisture better to gain a high yield. However, not all genotypes respond equally to improving conditions and can increase their productivity. Therefore, it is of scientific and practical importance to study the morphophysiological traits of winter bread wheat during the normal water supply in the late period under rainfed conditions without a stable moisture supply.

MATERIALS AND METHODS

The research was carried out at the Gobustan Regional Experimental Station (GRES) of the Research Institute of Crop Husbandry. The experimental site is located at an altitude of 800.0 m above sea level, and the soil cover belongs to the open chestnut soil type. According to average multi-year data, the amount of atmospheric precipitation in the region is 350.0-400.0 mm. 12 varieties and 9 lines of winter bread wheat, which differ in morphophysiological traits, were taken as the research objects. The area of each experimental bed was 1.0 m^2, and planting was performed in 3 replicates in the form of randomly placed blocks, and the seeding rate was 450 seeds per 1 m^2. At the beginning of May, artificial drought conditions were created by covering one block with a transparent polyethylene cover, and the second block was irrigated. Before harvesting, the height of 10 plants in each block was measured and the average height (cm) of the genotype was found. After completing the development, the width and length of the flag leaf of 10 plants were measured from the middle part (Kalaycı et al., 1998). The area of the leaf was calculated by multiplying the product of the width by the length by a factor of 0.72.

Relative water content (RWC) was determined in mature flag leaves (Turner, et. al., 2001). For this, the samples were taken at the hottest time of the day (between 14^{00} and 15^{00} hours). The stay green (SG) period of flag leaves was determined in Growing Degree Days (GDD) based on SPAD values (total chlorophyll content) measured on different dates (Choelho and Dale, 1980). The parameters of photosynthetic gas exchange - photosynthetic rate - P_n, stomatal conductance - g_s, and transpiration rate - T_r were measured using a LI-COR 6400 XT (LI-Cor Biosciences, Lincoln, USA) Portable Photosynthesis System equipped with a 6 cm^2 leaf chamber (Sharkey, 2016). The sheaves from every 3 repetitions (1 m^2) of the variants were threshed and weighed on a scale. The average value for three replicates was calculated and the obtained result was taken as productivity per 1 m^2 (g/m^2). Statistical analyses were performed in JMP 5.0.1 software.

RESULTS AND DISCUSSION

It is observed that the drought usually occurs after earing under rainfed conditions not provided with stable moisture. Therefore, some morphological and physiological characteristics

and productivity of winter bread wheat under conditions of water supply improvement and water shortage in the late period were studied and the relationship between them was considered. The results of the study of plant height, flag leaf area, leaf relative water content, the stay-green period of the flag leaf, and photosynthetic gas exchange parameters, which are important morphological and physiological parameters of wheat, are given in Tables 1 and 2. As seen in Table 1, the average plant height for all genotypes was 98.0 and 105.6 cm under conditions of water scarcity and normal water supply, respectively. During the improvement of water supply, the increase in plant height was 7.8%, which is slightly more compared to the drought-exposed variant. The growth in plant height was by 11.4, 10.6, 10.0, and 10.5%, respectively, in the Aran, Gyrmyzy gul 1, 12thIWWYT №8, and 7thWON-SA №477 genotypes, which was more compared to other studied genotypes. Gyzyl bughda, Sheki 1, Sonmez 01, Murov 2, Gobustan, Zirva 85, 7thWON-SA №465, and 12thIWWYT № showed a lower growth. The obtained results showed that the improvement of water supply in the late period increased the plant height of the early-earing genotypes less than that of the late-earing genotypes. The area of the flag leaf was 19.1 and 22.7 cm^2 on average for all genotypes during water shortage and improvement of water supply in the late period. In both research variants, the flag leaf area of the genotypes Bezostaya 1, Sheki 1, Aran, 12thIWWYT №9, and 7thWON-SA №477 was larger compared to that of Fatima, Gyrmyzy gul 1, Ferrigineum 2/19, and 12thIWWYT №6. Under the conditions of improved water supply, the area of the flag leaf of the Tale 38, Fatima, and Gyrmyzy gul 1 varieties increased by 24.8, 27.4, and 26.6% respectively, being higher than that of the other genotypes. We believe this feature creates better conditions for grain filling due to the greater increase in the area of photosynthesis during the improvement of water supply in the late period.

Table 1. Some morphological and physiological parameters of wheat genotypes under different water supplies

Genotypes	Plant height, cm		Flag leaf area, cm^2		RWC, %		Stay-green period, GDD	
	drought	irrigation	drought	irrigation	drought	irrigation	drought	irrigation
Bezostaya 1	106.3	115.8	24.5	27.3	77.4	84.0	460	528
Gyzyl bughda	106.0	111.8	21.6	25.1	66.8	69.2	469	560
Sheki 1	114.8	121.7	22.3	26.2	76.1	85.6	478	548
Sonmez 01	114.5	121.7	17.6	20.2	72.7	77.0	501	582
Aran	84.2	93.8	23.2	24.9	82.6	89.2	479	570
Vostorg	75.1	81.8	18.9	21.4	85.6	90.9	468	534
Murov 2	96.5	102.3	19.7	22.7	83.5	86.6	587	662
Gobustan	97.9	104.2	18.9	21.8	76.0	83.5	466	583
Tale 38	89.2	96.7	21.4	26.7	83.4	88.2	469	549
Fatima	93.0	99.3	14.6	18.6	66.3	73.2	480	582
Gyrmyzy gul 1	75.7	83.7	12.8	16.2	79.1	88.8	532	630
Zirva 85	91.0	96.7	19.0	23.2	75.4	83.8	557	657
7thWONSA№465	101.5	108.0	17.3	20.7	73.6	80.9	487	597
Ferrigineum 2/19	89.4	97.5	12.1	14.8	70.7	72.8	486	577
11thIWWYT№20	104.8	113.0	18.7	23.0	69.4	86.3	490	573
12thIWWYT№ 6	106.3	113.0	13.5	15.9	59.1	69.2	437	559
12thIWWYT№ 8	93.2	102.5	18.8	21.8	72.5	85.7	469	580
12thIWWYT№ 9	101.8	111.0	23.4	28.1	63.7	86.7	443	522
12thIWWYT№ 17	109.7	118.3	21.8	27.1	60.1	67.4	420	535
7thWON-SA №477	108.0	119.3	26.0	31.4	67.7	71.3	504	586
4th FEFWSN №50	97.5	104.5	17.9	22.3	69.5	83.5	476	554
Average	**98.0**	**105.6**	**19.1**	**22.7**	**72.9**	**81.1**	**484**	**575**
LSD	**4.6****	**5.0****	**0.95****	**1.2****	**5.42****	**5.5****	**34.0***	**40.0***
CV, %	**3.2**	**3.2**	**6.1**	**5.8**	**5.9**	**5.4**	**6.5**	**7.1**

Note: Relative water content (RWC), Growing Degree Days (GDD), LSD-least significant difference, CV (%) – coefficient of variation, **-significant at the 0.01 level, *- significant at the 0.05 level.

Table 2. Photosynthetic gas exchange parameters

Genotypes	P_n µmol CO_2 $m^{-2}s^{-1}$		g_s mol H_2O $m^{-2}s^{-1}$		T_r mmol H_2O $m^{-2}s^{-1}$	
	drought	irrigation	drought	irrigation	drought	irrigation
Bezostaya 1	13.0	19.8	0.096	0.353	2.77	7.48
Gyzyl bughda	11.2	22.9	0.077	0.456	2.26	8.33
Sheki 1	14.1	23.7	0.108	0.353	3.16	7.74
Sonmez 01	11.8	21.4	0.083	0.404	2.21	6.76
Aran	16.6	22.2	0.128	0.381	3.50	7.05
Vostorg	14.0	17.9	0.137	0.429	3.54	7.69
Murov 2	11.8	22.2	0.094	0.362	2.63	7.07
Gobustan	9.8	15.6	0.074	0.278	2.46	6.03
Tale 38	17.1	23.9	0.199	0.480	5.06	9.06
Fatima	13.8	19.0	0.147	0.397	2.74	6.68
Gyrmyzy gul 1	15.8	24.3	0.145	0.517	2.64d.	8.07
Zirva 85	12.1	18.9	0.130	0.429	2.63	7.08
7thWON-SA №465	8.1	20.9	0.062	0.451	1.52	7.59
Ferrigineum 2/19	10.6	20.3	0.069	0.395	1.86	7.08
11thIWWYT №20	14.0	23.7	0.200	0.442	3.20	8.06
12thIWWYT №6	9.7	21.8	0.078	0.372	2.16	7.67
12thIWWYT №8	13.0	21.3	0.096	0.422	2.76	7.44
12thIWWYT №9	11.8	19.3	0.088	0.255	2.52	5.77
12thIWWYT №17	9.7	23.1	0.060	0.363	1.65	7.08
7thWON-SA №477	8.8	22.3	0.079	0.438	2.02	7.55
4th FEFWSN №50	8.9	18.3	0.086	0.312	2.14	6.16
Average	**12.1**	**21.1**	**0.103**	**0.395**	**2.58**	**7.31**
LSD	**0.78****	**1.05****	**0.0096****	**0.0301****	**0.27****	**0.52****
CV, %	**5.7**	**4.4**	**8.2**	**6.5**	**9**	**6.2**

Note: P_n- photosynthetic rate, g_s - stomatal conductance - g_s, T_r - transpiration rate - T_r. LSD-least significant difference, CV (%) – coefficient of variation, **-significant at the 0.01 level, *- significant at the 0.05 level.

The relative water content of the flag leaf was 72.9% and 81.1% in drought and irrigation variants for all genotypes. The relative water content of the flag leaf in the varieties Aran, Vostorg, Murov 2, Zirva 85, Tale 38, and Gyrmyzy gul 1 was higher than in other studied genotypes under both drought stress and normal water supply conditions.

The study of the stay-green period of the flag leaf, which is one of the physiological parameters that play an important role in the grain filling and productivity of winter wheat, showed that its average value for all genotypes was 484 GDD under drought stress conditions, and 575 GDD under normal water supply conditions. The value of this parameter in Murov 2 and Zirva 85 varieties was higher than in other studied genotypes (Table 1). The relative water content of the flag leaf in the mentioned genotypes was also higher, which indicates that it is appropriate to use those genotypes in the hybridization process.

Carbon dioxide (CO_2) assimilation rate (P_n), stomatal conductance (g_s), and transpiration rate (T_r), which are the parameters of photosynthetic gas exchange, were measured between 11^{00}-12^{00} in the flag leaf during the milk ripeness phase in drought and irrigation variants. The results of the measurements are given in Table 2. The analysis of variance showed that there were significant differences between the studied genotypes at the 0.01 level of significance in all measurements for the values of photosynthetic gas exchange parameters. As seen in Table 2, the average values of CO_2 assimilation rate, stomatal conductance, and transpiration rate for all genotypes under drought and irrigated conditions were 12.1 and 21.1 µmol CO_2 $m^{-2}s$-1; 0.103 and 0.395 mol H_2O $m^{-2}s^{-1}$; 2.58 and 7.31 mmol H_2O $m^{-2}s^{-1}$, respectively.

The highest values of P_n were found in the varieties Tale 38, Aran, and Gyrmyzy gul 1 in both drought and irrigation variants. It should be noted that the highest values of the stomatal conductance in both variants were detected in the Tale 38 and Gyrmyzy gul 1 genotypes. The rate of transpiration was the highest in the Tale 38 variety in both drought and irrigation variants and was 5.06 and 9.06 mmol H_2O $m^{-2}s^{-1}$, respectively.

As seen, the rate of photosynthesis, stomatal conductance, and transpiration rate were higher in the irrigation variant than in the drought variant, which was the result of the declining water content in the soil below the norm.

As a result of the research, it was found that because the stomatal conductance was more sensitive to water deficit, its value was on average 73.9% lower in the drought variant compared to that of the irrigation variant. The most differences between variants were observed in the genotypes Gyzyl bughda, Gyrmyzy gul 1, 7thWON-SA №465, Ferrigineum 2/19, 12thIWWYT №17, and 7thWON-SA №477, respectively, 83.1, 87.4, 86.3, 82.5, 83.5 and 82.5%, and the least differences were found in the genotypes Aran (66.4%), Tale 38 (58.5%), Fatima (63.0%), 11 IWWYT №20 (54.8%), and 12 IWWYT №9 (65.5%). It was observed that the genotypes with high values of stomatal conductance also had high values of T_r, which indicates that T_r is mainly regulated by stomatal conductance. Thus, the study of photosynthetic gas exchange parameters showed differences between genotypes. At the same time, considering a higher value of this parameter as well as other superior morphophysiological traits, it was concluded that the Gyrmyzy gul 1 variety is appropriate for the hybridization program.

It is important to study the productivity of winter wheat genotypes under different water supply conditions. In Figure 1, the productivity of studied genotypes is given according to the average of two years. As seen in the Figure, the average productivity of all genotypes in the drought variant was 496 g/m^2, and in the irrigation variant, it was 623 g/m^2.

The highest productivity (607 g/m^2) in the drought variant was observed in the 7WON-SA № 465 line. Gobustan and Ferrigineum 2/19 genotypes followed it with 557 and 549 g/m^2. In the drought variant, the lowest productivity was detected in the Bezostaya 1, Vostorg, and Gyzyl bughda varieties (435, 438, and 441 g/m^2, respectively). In the irrigation variant, the highest productivity was 728, 717, 707, and 706 g/m^2, in the genotypes 7WON-SA № 465, Tale 38, Gyrmyzy gul 1, and Gobustan, respectively.

Due to the lodging of irrigation variants of the 12thIWWYT № 9 and 12thIWWYT №17 varieties, if their results are not taken into account, the average reduction of productivity for all genotypes in the drought variant compared to irrigation was 20.0% (Figure 2).

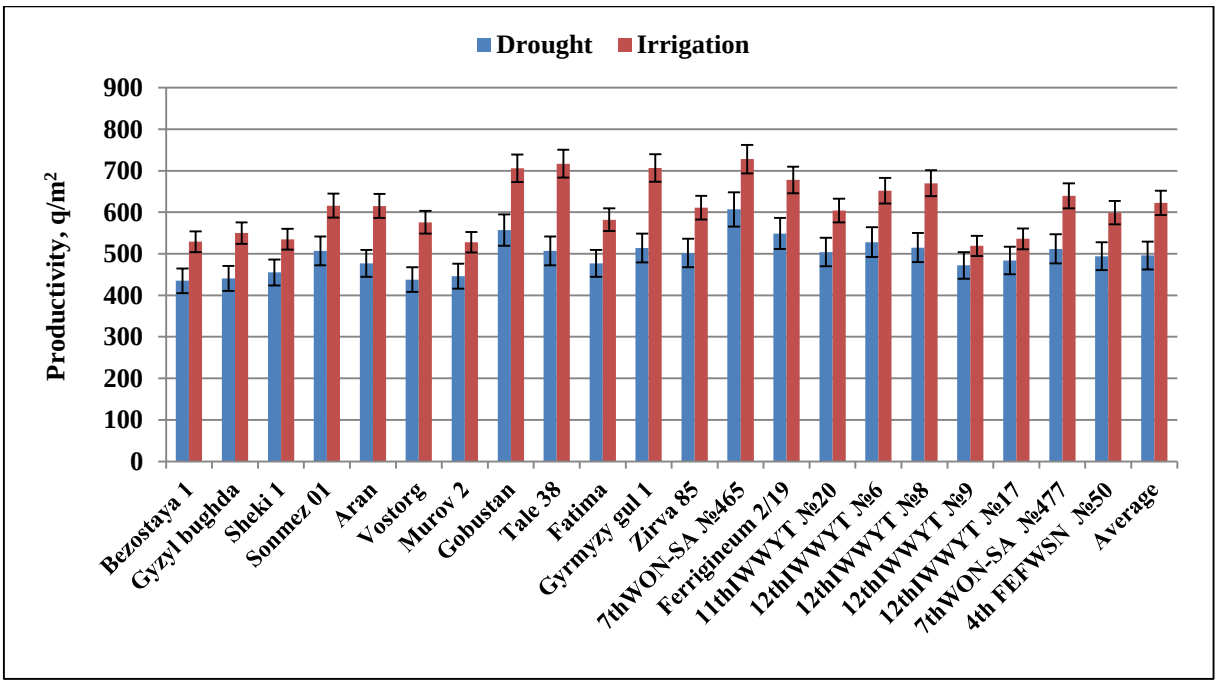

Fig. 1. Productivity of drought-exposed and irrigated genotypes.

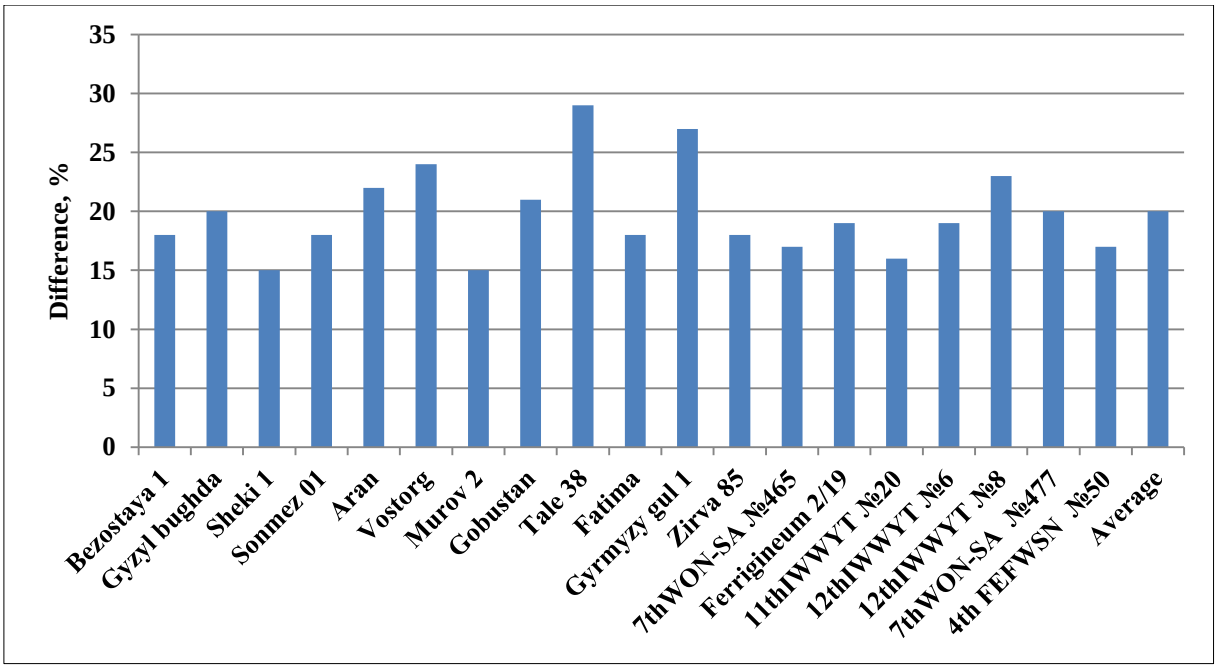

Fig. 2. Differences in the productivity of irrigation and drought variants, %.

Among the variants, the greatest reduction in productivity was observed in the varieties Tale 38 (29.3%), Gyrmyzy gul 1 (27.3%), Vostorg (24.0%) and in the line 12thIWWYT № 8 (23.1%), and the smallest reduction was observed in the genotypes Sheki 1 (15.0), Murov 2 (15.57 WON-SA №465 (16.6%), and 11thIWWYT№ 20 (16.6%).

As seen in Figure 1, the productivity of the genotypes Tale 38, Gyrmyzy gul 1, and 12thIWWYT № 8 in the drought variant was close to the average level, and in the irrigation variant, it was much higher than the average level. The fact that those genotypes respond more to optimal climatic conditions and increase their productivity more than other genotypes is an indication of their high plasticity. Sheki 1, Murov 2, and 7WON-SA № 465 had a low productivity reduction in the drought variant, which, we believe, indicates that their stability is higher than that of other genotypes.

CONCLUSIONS

Thus, the study of the effect of the improvement of water supply in the late period on some morphophysiological traits of winter bread wheat under rainfed conditions without a stable moisture supply showed that it had a small effect on the plant height, and a greater effect on the area of the flag leaf, relative water content, and the stay-green period of the flag leaf. Genotypic differences were observed in the values of the studied parameters. The higher relative water content of the flag leaf and the stay-green period of the genotypes Gyrmyzy gul 1, Murov 2, and Zirva 85 confirmed their relevance for the hybridization in the breeding program of winter wheat. The Gobustan variety and the line 7thWONSA №465 had the highest productivity under drought stress conditions. Since other morphophysiological parameters of those genotypes were also favorable, it was recommended to plant them in rainfed areas without a stable moisture supply. The Tale 38 and Gyrmyzy gul 1 varieties were found to have high potential, which showed their relevance for planting under irrigated conditions. It was found that stomatal conductance is more sensitive to water deficit, the rate of transpiration and photosynthesis depends on stomatal conductance, and at the same time, the rate of transpiration is more sensitive to stomatal conductance than the rate of photosynthesis.

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Evaluation of wheat genotypes according to morphophysiological and economic value characteristics under drought conditions

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The main goal of the research was a comparative assessment of zoned genotypes of durum and bread wheat, under drought conditions, according to morphophysiological and economically valuable traits, grain quality indicators, determining the correlation between them and their use in breeding. It has been established that plants, due to increased temperature and accelerated transpiration in the afternoon, are more susceptible to the effects of drought. From this point of view, the values of daily water deficit according to the experimental variants varied in a wide range at the heading phase - the formation of wheat grains. In contrast to the daytime water deficit, all varieties, regardless of water availability, restored the missing water in the plant by reducing transpiration at night. This pattern was confirmed by the results obtained from the residual water deficit of flag leaves. The values of water-holding capacity, which is an indicator of plant resistance to drought, in flag leaves of wheat genotypes, were high mainly under drought conditions, regardless of the biological characteristics of the varieties. Based on many years of research, it has been established that the area of flag leaves in plants reaches its maximum size in the heading-flowering phase. In the studied genotypes, the area of flag leaves varied within the range of 15.8-40.7 cm^2 under irrigation, 12.5-36.0 cm^2 under drought, the difference between the variants ranged from 10.0% to 43.8%. The specific surface density of flag leaves in wheat varieties, as an important photosynthetic trait, varied over a wide range among the studied varieties. In wheat genotypes with high values, the specific surface density of leaves in both variants led to high grain yield per unit area. This led to high grain yield per unit area in both options. In general, the size of the grain yield due to drought in the studied varieties decreased by 16.5-38.2%. The bread wheat genotypes Gyrmyzy gul 1, Jumhuriyet 100 and Khazri with high architectonics, grain yield (732-746 g/m^2 and 477-538 g/m^2), were distinguished in both variants by their short stature, better restoration of lost water at night, and optimal area sizes flag leaf (15.8-25.9 cm^2 and 12.5-23.3 cm^2) and high values of the specific surface density of flag leaves (0.408-0.777 g/dm^2 and 0.509-0.596 g/dm^2), low values of length from flag leaf of the spike (11.5-15.9 cm and 7.7-12.6 cm), the optimal length of the last internode (15.3-19.0 cm and 15.4-18.5 cm), with small distances from the last node to the spike (28.2-33.4 cm and 23.6-29.8 cm), high protein yields (85.6-91.8 g/m^2 and 62.8-69.4 g/m^2) per unit area. Correlations between morphophysiological and economically valuable traits of wheat differed under drought conditions. Similar positive and negative correlations between the studied traits under drought conditions were also obtained in the irrigated variant. However, in contrast to the water-supplied variant of plants, under drought conditions, positive and negative correlations were obtained between the WHC of the flag leaf with TWC (r=0.680), LLIN with LLNS (r=0.642*), YI with GY (r=0.667*), amount of protein in grain with TWC (r=0.873), protein yield per unit area and GY(r= 0.967**), MI (r=0.609*), yield index and LLNS (r=-0.629).**

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Keywords: *Drought, wheat, genotype, water regime, morphophysiological characteristics, yield, yield index, quality, correlation dependencies*

INTRODUCTION

Today, water shortage is considered one of the main pressing problems facing agriculture. The critical situation in meeting the irrigation water needs of the population and agriculture has worsened, primarily due to climate change. The land and water resources that support agricultural production and food security for the world's population are under threat. Agriculture currently uses 11% of the world's land and 70% of freshwater resources for agricultural production (FAO, 2012).In this regard, the annual volume of total surface water resources, which has become one of the decisive factors for the sustainable development of the agricultural sector of Azerbaijan, is 30-31 cubic km. In dry years, this reserve decreases to 20.3 cubic km. Two-thirds of our water supplies are generated outside the country. Therefore, Azerbaijan did not remain aloof from these risks. All this requires the study of the morphophysiological, biochemical and agronomic principles of creating drought-resistant plant varieties for arid regions, as well as the use of technologies that save water resources in agriculture.

In 2019-2023, the total area under wheat, considered strategic for the country, fluctuated between 572.4-672.2 thousand hectares. Over the past five years, the total volume of wheat production in Azerbaijan amounted to 1736.1-2171.5 thousand tons, the average yield was 3.17-3.29 tons/ha. This allows us to cover up to 60% of our wheat needs in the country. Since up to 40% of wheat crops fall in the rain-fed regions of the country. Drought, which often occurs during the reproductive phase of wheat growing season in rain-fed areas, causes a decline in overall wheat production. To obtain a high yield in the rainfed regions of the country, it is necessary to use highly reproductive, certified seeds of drought-resistant wheat genotypes, soil and moisture protection technologies. Effective breeding is based on a comprehensive study of morphophysiological and economically valuable traits and the creation of new wheat genotypes on this basis. Therefore, genotypes containing the results of a multidisciplinary comprehensive assessment are considered competitive (Maimistov, 2000; Talai, 2010). Currently, numerous studies conducted using the comparative method under irrigation and drought conditions have established the presence of correlations between morphophysiological and economically valuable traits when studying the photosynthetic activity of wheat (Aliev et al., 1988; Jahangirov, 2023; Jahangirov et al., 2022). In this regard, drought stress is considered one of the main factors limiting wheat production in the world. One of the main reasons for the decrease in winter wheat yield is an increase in air temperature and lack of moisture in the soil, as a result of which the normal course of physiological and biochemical processes in plants is disrupted. Studying the water regime, morphophysiological and economically valuable characteristics of wheat genotypes under drought conditions will allow us to draw conclusions about their resistance to drought (Tamrazov, 2021). When determining a wheat breeding strategy under drought conditions, many researchers considered it advisable to evaluate germplasm both in near-optimal and under stress conditions (to protect drought-resistant alleles) (Bauder, 2001; Balota et al., 2007).

Drought stress affects many morphological and physiological parameters of winter wheat. To increase the efficiency of the selection of drought-resistant varieties, it is necessary to select indicators that can accurately show the drought resistance of the variety. Many researchers, when assessing wheat for drought resistance, considered it important to study plant height, structural elements of the crop, the amount of water in the leaf, photosynthetic traits, photosynthesis intensity, CO_2 concentration in the intercellular region, peroxidase activity and the amount of abscisic acid (Xiaoyuan et al., 2023; Bardees et al., 2020). The study of water regime indicators, including the water-holding capacity of leaves of winter wheat genotypes during drought, is of great practical importance. Genotypes of common wheat resistant to water stress were distinguished by high values of the water-holding capacity of leaf tissues

during the heading-flowering phase (Nekrasov et al., 2020). The water-holding capacity of leaves is positively related to the drought resistance of the variety under field conditions and economically valuable traits, which is especially noticeable in dry years. The maximum values of grain weight in the main ear, lateral stems and the plant as a whole were formed in those varieties that have a slight loss of moisture during wilting (Sanina, 1996). On days of high temperatures (May, June), a sharp increase in the intensity of transpiration leads to an imbalance in the water balance in the wheat plant and, as a consequence, to a lack of water. One of the signs of this is a sharp increase in water shortage in the second half of the day, which is accompanied by a decrease in the rate of CO_2 assimilation during photosynthesis. Of course, all this does not go unnoticed by the plant and is manifested by disruption and retardation of growth and reproductive processes, all of which ultimately lead to a decrease in plant productivity (Ergashev et al., 2010).

The main goal of the study was to compare varieties of durum and bread wheat, zoned under drought conditions, according to morphophysiological and economically valuable characteristics, grain quality indicators, to determine the relationships between them and their use in breeding. Based on the purpose of the research, the tasks ahead include studying the effect of drought on water regime indicators, morphophysiological characteristics, yield, harvest index, amount of protein in grain and protein yield per unit area of wheat varieties.

MATERIALS AND METHODS

Field experiments to achieve this goal were carried out in the fall of 2020-2022. at optimal sowing dates (between the third ten days of October and the first ten days of November) and the norm (200 kg/ha) at the Absheron experimental base of the Research Institute of Agriculture. Field experiments were carried out in 4 replicates on an area of 25 sq. m. The site where the experimental field is located on Absheron is located in circles N 40º31. 957ʿ latitude and E 49º52. 525ʿ longitude, at an altitude of 6 m above sea level. The climate of Absheron is predominantly moderate, hot and dry, with favorable conditions. This is a windy and low-precipitation (200-400 mm) region of the republic, the average annual air temperature is 13.9ºC. The common gray-brown type of soil in Absheron has alkaline properties, carbonate, the amount of total humus in the arable layer is low and amounts to 1.27-1.32%. As the object of study, 3 genotypes of durum and 9 bread wheat were used, zoned and belonging to the selection of the Scientific Research Institute of Crop Husbandry. In the studied wheat genotypes, the angle of deviation of flag leaves from the stem ranged from 10-80º, and in the bread wheat varieties Tale 38, Girmyzy gul 1 and Jumhuriyet 100 with high architectonics and vertically oriented leaves, it ranged from 10-20º. The tetra- and hexaploid wheat genotypes were studied in irrigated (control) and arid (experimental) variants and optimal fertilizer rates (N_{90}, P_{90}, K_{60}). In the control variant, the plants were watered 3-4 times depending on the year of vegetation. In field experiments, the dynamics of moisture distribution in soil layers of 0-20, 20-40 and 40-60 cm (Lysogorov et al.,1985), the development phases of winter wheat and the stages of organogenesis were determined using the thermostat-weight method, were determined using the Fix and Zadox scales (Large,1954). The water regime of flag leaves, including total water capacity (TWC), daily water deficit (DWD) and residual water deficit (RWD), water holding capacity (WHC) based on the guidelines of the All-Russian Institute of Plant Genetic Resources named after N.I.Vavilov (Borodina,1987), the areas of flag leaves were determined using an automatic leaf area meter "AAS-400", manufactured in Japan, and the specific surface density of the leaf (SSLD) was calculated as the ratio of the biomass of leaf samples to its area. Productivity was calculated based on the weight of grain in sheaves taken from 1 sq. m. The amount of total nitrogen was determined by the modified Keldahl micromethod using a Kjeltec TM8200 instrument manufactured by FOSS (Pleshkov, 1976). To calculate the amount of protein, the coefficient Nx 5.70 was used. The amount of raw gluten corresponds to state standards (Sozinov, 1977), the yield index was calculated as the ratio of grain to plant biological mass. Correlation dependencies between morphophysiological and economically valuable traits were determined using the statistical program SPSS 16. 0.

RESULTS AND DISCUSSION

During the years of research, the dynamics of moisture distribution in different soil layers were studied, starting from the 1st ten days of March to June. In wheat, this period covers from the end of the spring tillering phase to the phase of waxy ripeness of the grain. Depending on meteorological and growing conditions, the distribution of moisture in soil layers during the growing season varied depending on a number of factors, including the water supply of crops. Optimal soil moisture for most crops, including wheat, should be 65-85% of the minimum field soil moisture capacity. In this regard, the experimental plot in the control version was watered to maintain soil moisture at a given level during the growing season. During the tillering phase, the plants' need for water is minimal; starting from the tubing phase, it begins to increase due to the intensive formation of above-ground biomass. No significant differences were observed between the treatments at the end of the tillering phase and at the beginning of booting. Therefore, starting from the end of the tubing phase, the difference between the variants in soil moisture begins to increase due to a gradual increase in air temperature and irrigation of the experimental plot in the control variant. In general, the average soil moisture during the growing season in the control variant ranged from 78.7-57.8%, and in the experimental variant – 59.7-31.8% based on the lowest field moisture capacity. As you can see, the plants cultivated in the experimental version, especially in the reproductive period of the growing season, are in unfavorable conditions. The role of water regimes in the drought resistance of biological systems is irreplaceable. Since the physiological processes occurring in plants largely depend on the amount and state of water in cells and tissues. All this, in turn, determines the direction and intensity of these processes. In this regard, when determining the response of wheat genotypes to different water regimes, it is of great importance to study the indicators of different tiers of leaves, other vegetative and generative organs in different phases of vegetation development. Therefore, studying the indicators of the water regime of plants under drought conditions allows us to formulate ideas about the drought resistance of wheat varieties (Clarke et al.,1982; Rustamov et al., 2012).

During the years of research, some indicators of the water regime, including total water capacity, daily water deficit, residual water deficit and water-holding capacity in the flag leaf tissues of durum and bread wheat genotypes, were comparatively studied depending on water availability during the heading-formation period of grain.

High values of total water capacity in leaf tissues of wheat genotypes under drought conditions had a positive effect on the course of physiological processes in plants. In the studied genotypes of durum and bread wheat during the period of heading-grain formation, the TWC values in flag leaves in the control variant ranged from 66.8-72.5%, and under drought conditions 63.6-70.8%. At the reproductive stage of the growing season in the control variant, the maximum TWC values at noon were observed in the wheat genotypes Garagylchyg 2, Giymatli 2/17, Gyrmyzy bugda, Deyirman and varied between 71.3-72.5%. At noon, the difference between the options according to TWC data was 1.7-3.2%. Compared to the control variant under drought conditions, minimal decreases in the TWC value in flag leaves at noon were recorded in the wheat genotypes Gyrmyzy bugda, Barakatli 95, Khazri, Nurlu 99, Jumhuriyet 100, Saratovskaya 29, and the maximum values were recorded in the genotypes Deyirman, Giymatli 2/17, which amounted to 0.7-3.3% and 5.5-6.8%, respectively. The tall wheat genotype Gyrmyzy bugda differed in both variants in its maximum TWC values (Fig.).

Plants, as a result of rising temperatures and accelerated transpiration in the afternoon, are more susceptible to the effects of soil and atmospheric drought. From this point of view, the daily water deficit was determined in flag leaves during the period of heading and grain formation. DWD expresses the amount of water missing in plant tissues due to soil and atmospheric drought. During this growing season, the DWD values according to the experimental variants varied in a wide range and amounted to 17.4-24.9% and 19.3-30.7%, respectively.

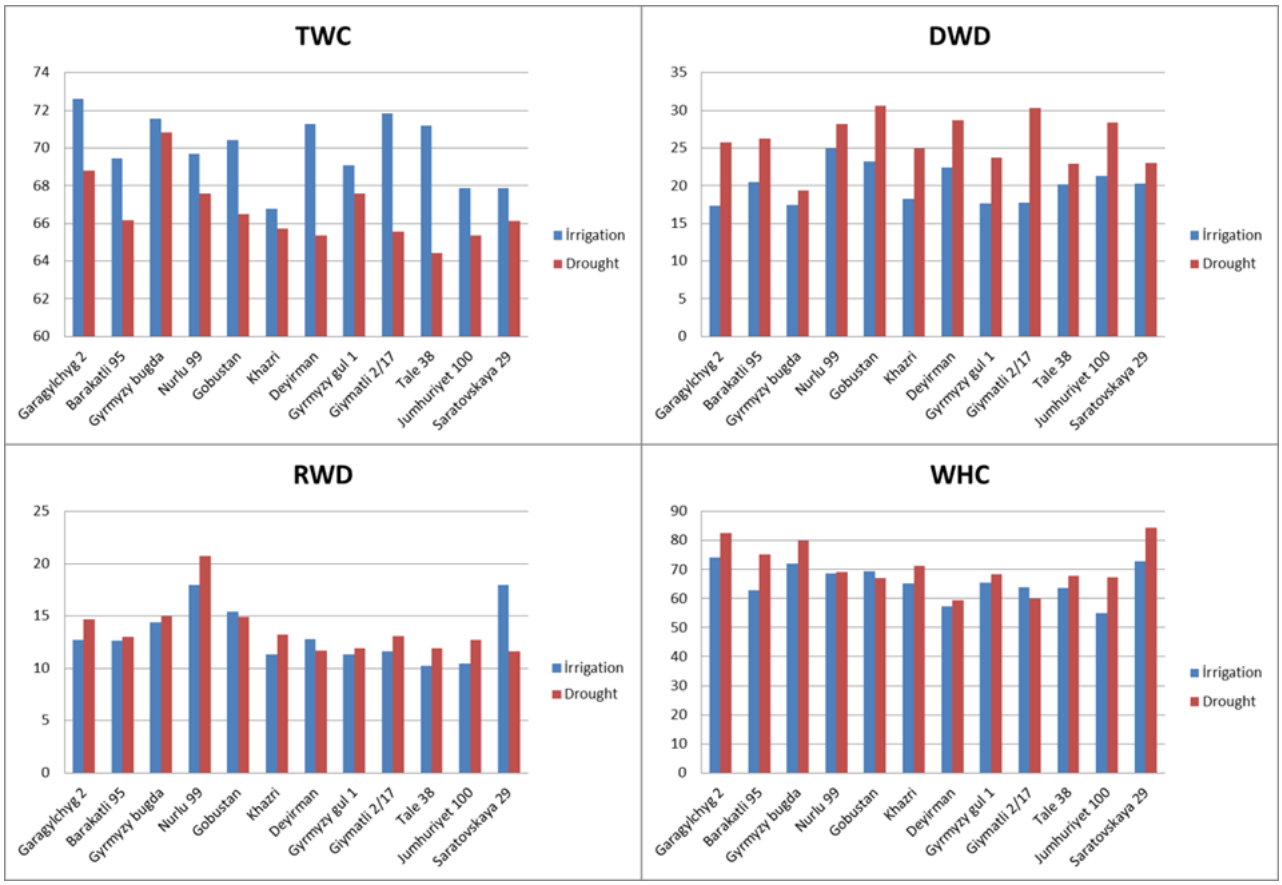

Fig. Some indicators of the water regime of flag leaves of durum and bread wheat varieties depending on water availability, % (Absheron, average indicators 2021-2023); ***Note:*** TWC - total water capacity, DWD - daily water deficit, RWD - residual water deficit, WHC - water holding capacity

In the experimental version, since the plants suffered from drought, this led to high values of DWD in flag leaves; this difference ranged from 0.6-16.6% depending on the biological characteristics of the genotypes studied. The highest rates of DWD during drought were observed in the genotypes Gobustan (30.7%), Giymatli 2/17 (30.3%), Deyirman (28.7%), Nurlu 99 (28.2%), and the lowest values in Gyrmyzy bugda (19.3%), Tale 38 (22.9%), Saratovskaya 29 (23.0%), Gyrmyzy gul 1 (23.7%) (Figure).

All genotypes, in contrast to DWD, restored the missing water in the plant by reducing transpiration at night. This pattern was confirmed by the results obtained from the residual water deficit of flag leaves. Therefore, RWD is considered the most reliable and objective indicator of drought resistance. The water supply is set early in the morning, before sunrise, so at this time transpiration is reduced to a minimum and reflects the lack of water that plants cannot recover in the evening. Indicators of RWD in leaves of the studied wheat genotypes were 10.2-17.9% in the irrigated variant and 11.6-20.8% under drought conditions. Plants grown under irrigated conditions were relatively better at restoring the missing water at night. This is due to the regulation of soil moisture according to the lowest field moisture capacity and the strong development of the root system in the control variant. Under drought conditions, the common wheat genotypes Saratovskaya 29, Deyirman, Gyrmyzy gul 1, Tale38, Jumhuriyet 100, Khazri worked effectively, better restoring the missing water at night, and the water content values fluctuated between 11.6-13.3% (Figure).

Table 1. Morphophysiological characteristics of durum and bread wheat genotypes depending on water availability (Absheron, average for 2021-2023)

Genotype name	Variant of experience	Flag leaf area, cm^2	Specific surface density of the flag leaf, g/dm^2	Length from the flag leaf of the spike, cm	Length of the last internode, cm	Length from the last node to the spike, cm
Durum wheats						
Garagylchyg 2	irrigation	25.0	0.534	20.1	14.5	42.0
	drought	15.5	0.430	13.7	15.7	31.9
Barakatli 95	irrigation	23.6	0.524	15.2	15.6	36.2
	drought	17.7	0.517	11.1	12.8	30.2
Gyrmyzy bugda	irrigation	33.3	0.388	33.1	25.5	60.7
	drought	27.1	0.388	17.5	21.7	44.7
Bread wheats						
Nurlu 99	irrigation	23.8	0.449	20.5	14.8	30.5
	drought	18.4	0.528	18.4	16.0	35.5
Gobustan	irrigation	25.5	0.421	20.2	19.7	42.1
	drought	21.6	0.536	18.6	16.4	40.7
Khazri	irrigation	25.9	0.508	15.9	19.0	33.4
	drought	23.3	0.509	12.6	18.5	29.8
Deyirman	irrigation	34.0	0.594	16.0	16.2	32.3
	drought	19.1	0.316	12.6	14.8	31.8
Gyrmyzy gul 1	irrigation	15.8	0.777	13.6	15.3	31.1
	drought	12.5	0.596	7.70	15.4	23.6
Giymatli 2/17	irrigation	40.7	0.388	16.0	17.5	35.9
	drought	36.0	0.269	8.00	14.8	26.2
Tale 38	irrigation	35.4	0.464	24.1	16.3	46.8
	drought	30.4	0.448	19.5	16.7	39.6
Jumhuriyet 100	irrigation	20.6	0.675	11.5	19.0	28.2
	drought	16.4	0.566	10.5	17.6	28.2
Saratovskaya 29	irrigation	19.7	0.491	28.8	23.2	51.1
	drought	15.2	0.623	24.5	19.8	45.7

The water-holding capacity of leaves, being an indicator of plant resistance to drought, was high in the non-irrigated version, regardless of the biological characteristics of the genotypes. Because the WHC indicators of flag leaves in the control variant changed in the range of 54.9-74.2%, and in the experimental variant 59.4-84.4%. This difference was 4.5-10.2% compared to the control variant. Under drought conditions, the highest WHC values were observed in the genotypes Garagylchyg 2, Saratovskaya 29, Gyrmyzy bugda, Barakatli 95, and Khazri, the minimum values were noted in the genotypes Deyirman, Giymatli 2/17, which varied between 71.2-84.8 and 59.4-60.0% respectively. The remaining genotypes were in an intermediate position according to these indicators. However, minimal differences between the variants in terms of WHC values were observed in the genotypes Nurlu 99, Gobustan, Deyirman, and Gyrmyzy gul 1 (Figure).

The morphophysiological characteristics that determine the productivity of the studied wheat genotypes were once again confirmed in the studies conducted. Table 1 for the studied genotypes shows the maximum values of the flag leaf area (FLA), the specific surface density (SSD), length from the flag leaf of the spike (LFLS) and the length of the last internode (LLIN), the length from the last node to the spike (LLNS). In this aspect, a comparative study of the activity of photosynthesis under conditions of irrigation and drought, combining several important features, provided certain information about the role of leaves as an assimilation organ in the formation of the crop and its potential.

Based on many years of research, it has been established that the area of flag leaves in plants reaches its maximum size in ontogenesis during the heading-flowering period. In the studied genotypes, the area of flag leaves ranged from 15.8-40.7 cm^2 under irrigation and 12.5-36.0 cm^2 under drought, the difference between the variants

varied within 10.0-43.8%. In both variants, the wheat genotypes of the extensive type Gyrmyzy bugda (37.8 and 30.8 cm^2), Giymatli 2/17 (40.7 and 36.0 cm^2) distinguished themselves with the maximum sizes of flag leaves, with the minimum values Gyrmyzy gul 1 (15.8 and 12.5 cm^2), Jumhuriyet 100 (20.6 and 16.4 cm^2), Saratovskaya 29 (19.7 and 15.2 cm^2). The remaining varieties were in an intermediate position according to this indicator. The bread wheat genotypes Gyrmyzy gul 1, Jumhuriyet 100, Khazri, which have high architectonics and optimal leaf area sizes, were also distinguished by high grain yields. Similar results were obtained for wheat genotypes based on the specific surface density of flag leaves (SSD). These indicators for the experimental variants were 0.388-0.777 g/dm^2 and 0.269-0.623 g/dm^2. Under irrigated conditions, wheat varieties were characterized by predominantly high SSDL values. The difference between the variants in terms of LPL was 1.34-46.8 %. In the Gyrmyzy bugda variety, the size of the SSDL was the same in both variants. The smallest difference between the options was observed in Barakatli 95 and Tale 38 wheat, and the largest difference was noted in the Deyirman and Ghiymatli 2/17 wheat genotypes and ranged from 1.34-3.45% and 30.7-46.8%, respectively. In both variants, the maximum SSDL values were distinguished by the intensive type wheat Gyrmyzy gul 1 (0.777 and 0.596 g/dm^2), Jumhuriyet 100 (0.675 and 0.566 g/dm^2), the minimum genotypes of the extensive type Gyrmyzy bugda (0.388 g/dm^2) and Giymatli 2/17 (0.388 and 0.269 g/dm^2). High values of SSDL made it possible to more effectively use assimilation processes involved in leaf growth to form the assimilation surface area of wheat varieties (Table 1).

The length of the donor-acceptor path plays an important role in the transport of assimilates. Here, the size, length from the flag leaf of the spike (LFLS), is of great importance. There is an opinion that when it is short, rapid movement of assimilates from the leaves to the ear is ensured. At this time, the consumption of organic substances for respiration decreases, which leads to an increase in the accumulation of substances in the grain (4). However, in different genotypes, a positive correlation between the LFLS and the size of the yield is not always observed. In the studied genotypes, the LFLS ranged from 11.5-33.1 cm under watering and 7.7-24.5 cm under drought. The genotypes Gyrmyzy gul 1 (13.6 and 7.7 cm), Jumhuriyet 100 (11.5 and 10.5 cm) were distinguished by small values of spikelets in both studied variants; the maximum sizes were noted in wheat varieties of the extensive type, tall Gyrmyzy bugda (33.1 and 27.5 cm), Saratovskaya 29 (28.8 and 24.5 cm) (Table 1).

During the years of the research, plant height (PH), length of the last internode (LLIN) and length from the last node to the ear (LLNS), which are considered one of the main morphobiological characteristics of wheat varieties, have been comparatively studied. Many researchers note a high correlation between plant height and the length of the last internode and drought resistance, especially during the heading phase. Thus, the latter is very sensitive to unfavorable environmental conditions, especially drought. In the genotypes participating in the study, plant height during irrigation was 80.7-130.6 cm, LLIN - 15.4-25.5 cm, LLNS – 28.2-60.7 cm, and during drought, respectively, fluctuated within 76.5-121.3 cm, 14.5-21.7 cm and 28.2-45.7 cm. The short-stemmed group included the genotypes of wheat Gyrmyzy gul 1 (80.7 and 76.5 cm), Deyirman (83.3 and 74.2 cm), the tall group included the extensive type of wheat Gyrmyzy bugda (130.6 and 121.3 cm) and Saratovskaya 29 (120.6 and 108.7 cm). Similar results for the indicated genotypes were obtained when measuring LLIN and LLNS. The results obtained allowed us to conclude that there is a positive correlation between plant height and LLIN and LLNS. The depression of plant height under drought conditions, depending on the biological characteristics of the variety, was 1.8-13.5%, according to LLIN 0.65-17.9%, LLNS 1.5-27.0% (Table 2).

The grain yield (GY) of different wheat genotypes varies depending on the biological characteristics of the variety, soil and climatic conditions, meteorological factors of the growing season and water availability. Average long-term grain yields obtained at the Absheron experimental base, under irrigation conditions, amounted to 418-746 g/m^2, under drought conditions 319-538 g/m^2.

Table 2. Economically valuable characteristics of durum and bread wheat genotypes depending on water availability (Absheron, on average for 2021-2023)

Genotype name	Variant of experience	Plant height, cm	Grain yield, g/m^2	Yield index	1000 grain weight, g	Protein content in grain, %	Protein yield, g/m^2
Durum wheats							
Garagylchyg 2	irrigation	88.5	463	0.36	44.4	12.9	59.7
	drought	80.5	319	0.31	39.9	14.9	47.5
Barakatli 95	irrigation	92.3	524	0.34	49.4	12.3	64.5
	drought	87.7	324	0.28	43.8	13.0	42.1
Gyrmyzy bugda	irrigation	130.6	439	0.19	47.1	12.7	55.8
	drought	121.3	366	0.21	41.9	14.7	53.8
Bread wheats							
Nurlu 99	irrigation	89.1	486	0.34	39.3	12.8	62.2
	drought	77.1	375	0.34	33.2	13.9	52.1
Gobustan	irrigation	94.0	610	0.39	44.1	12.2	74.4
	drought	92.3	423	0.33	34.1	14.0	59.2
Khazri	irrigation	86.5	740	0.42	46.9	11.9	88.1
	drought	78.5	483	0.32	41.9	13.0	62.8
Deyirman	irrigation	83.3	595	0.36	53.2	12.3	73.2
	drought	74.2	449	0.35	46.4	12.9	57.9
Gyrmyzy gul 1	irrigation	80.7	746	0.43	39.0	12.3	91.8
	drought	76.5	538	0.40	35.4	12.9	69.4
Giymatli 2/17	irrigation	86.6	443	0.34	49.7	12.3	54.5
	drought	82.6	359	0.30	44.5	13.3	47.7
Tale 38	irrigation	95.9	603	0.35	43.9	12.1	72.9
	drought	89.6	473	0.31	39.1	13.2	62.4
Jumhuriyet 100	irrigation	88.6	732	0.42	43.7	11.7	85.6
	drought	83.5	477	0.35	38.4	13.5	64.4
Saratovska ya 29	irrigation	120.6	418	0.28	34.2	13.1	54.8
	drought	108.7	349	0.28	31.6	13.5	47.1

The grain yield of the common wheat genotypes Gyrmyzy gul 1, Khazri, Jumhuriyet 100, characterized by high architectonics, vertical orientation of leaves and optimal FLA sizes, lower values of RWD and high values of SSD of flag leaves, the short base of LFLS in both variants was high. The yield of these varieties ranged from 603 to 746 g/m^2 under irrigated conditions and 477-538 g/m^2 under drought conditions. In both options, the minimum yield values were obtained for the extensive wheat genotypes Gyrmyzy bugda, Garagylchyg 2, Saratovskaya 29 and Giymatli 2/17. For these genotypes, the average yield values for the options were 29.9 and 21.4% less than for other genotypes, respectively. This contrast in productivity indicators is also reflected in the yield index (YI) of wheat genotypes. Similar results were obtained on high-yielding varieties. The yield index, which expresses the ratio of grain yield and the amount of total biomass, under irrigation conditions was 0.19-0.43 and under drought – 0.21-0.40 for the studied genotypes. In both variants, the genotypes of bread wheat Girmyzy gul 1, Jumhuriyet 100 with high productivity also differed in terms of the yield index. In these genotypes, 42-43% of the organic mass collected during irrigation is used to form the crop, and 35-40% during drought. In both options, the lowest yield index values were obtained for the tall wheat genotypes Gyrmyzy bugda and Saratovskaya 29. Here, the share of the grain yield in the total biological harvest was 19-28 and 21-28%, respectively. Thus, the growth of dry biomass in genotypes of this type occurs mainly due to vegetative organs (Table 2).

The studied wheat genotypes also differed in 1000 grain weight (TGW) depending on growing conditions. The weight of 1000 grains, considered the main indicator of grain filling and size, was expressed in contrasting values in the genotypes studied. Thus, the weight of 1000 grains fluctuated between 34.2-53.2 g during irrigation and 31.6-46.4 g during drought. Under the influence of

drought, the weight of 1000 grains decreased to 12.1%. According to this indicator, the minimum differences between the variants were obtained in the Saratovskaya 29 (7.6%), Gyrmyzy Gul 1 (9.2%) genotypes, the maximum differences in the Gobustan genotype (22.7%) (Table 2).

Determining the biochemical parameters of grain is of great importance when characterizing the quality indicators of wheat genotypes depending on water availability. From this point of view, the studied genotypes differed sharply in protein content in grain(PCG) and protein yield(PY) per unit area under different growing conditions. The maximum values of the amount of protein in grain were obtained in the dry variant. Under irrigation, these figures were 11.7-13.1%, in dry conditions – 12.9-14.9%. Genotypes of durum wheat Gyrmyzy bugda, Garagylchyg 2, Gobustan, Nurlu 99, Jumhuriyet 100, Saratovskaya 29 bread wheat under drought conditions were distinguished by the highest levels (13.5-14.9%) of protein content in grain. The studied genotypes also differed in protein yield per unit area depending on cultivation conditions. The protein yield per unit area for irrigated wheat genotypes was 54.5-91.8 g/m^2, and during drought – 42.1-69.4 g/m^2. The highest yields of protein per unit area in both variants were distinguished by the high-yielding varieties Gyrmyzy gul 1, Jumhuriyet 100, Khazri, Tale 38, Gobustan. These indicators for the options were 72.9-91.8 g/m^2 and 59.2-69.4 g/m^2 (Table 2).

During the years of research, depending on the water supply, the correlations between the morphophysiological and economically valuable characteristics of wheat genotypes were comparatively studied.

Under irrigation conditions, highly significant positive correlations were recorded between plant height and the WHC of the flag leaf (r=0.694*), WHC and RWD (r=0.694*), FLA and TWC (r=0.641*), LFLS and WHC of the leaf (r=0.723* *), PH (r=0. 904**), LLNS and WHC of the flag leaf (r=0.654*), plant height (r=0. 898**) and LFLS (r=0.933**), GY and SSD of the flag leaf (r=0.595*), SBAU and PH (r=0.861), LFLS(r=0.666*), LLNS (r=0.705*), TGW and FLA (r=0.668) *), amount of protein in grain and WHC (r=0.735**), WHC(r=0.832**) and LFLS (r=0.640*). However, under these conditions, highly significant negative correlations were also recorded between the SSD of the flag leaf with the FLA (r=-0.753**), YI and PH (r=-0.875**), LFLS (r=-0.864**), LLNS (r=-0.824**), TGW and WHC of the flag leaf (r=-0.622*), protein content in grain and GY (r=-0.804**), YI (r=-0.630*), LLIN and YI (r=-0.621*).

Correlations between morphophysiological and economically valuable traits of wheat differed under drought conditions. Similar positive and negative correlations between the studied traits under drought conditions were also obtained in the irrigated variant. However, in contrast to the water-supplied variant of plants, under drought conditions, positive and negative correlations were obtained between the WHC of the flag leaf with TWC (r=0.680), LLIN with LLNS (r=0.642*), YI with GY (r=0.667*), amount of protein in grain with TWC (r=0.873**), protein yield per unit area and GY(r=0.967**), MITGW (r=0.609*), yield index and LLNS (r=-0.629).

CONCLUSIONS

Thus, the size of the grain yield due to drought in the studied genotypes decreased by 16. 5-38. 2%. The bread wheat genotypes Gyrmyzy gul 1, Jumhuriyet 100 and Khazri with high architectonics, grain yield (732-746 g/m^2 and 477-538 g/m^2), were distinguished in both variants by their short stature, better restoration of lost water at night, and optimal area sizes flag leaf (15.8-25.9 cm^2 and 12.5-23.3 cm^2) and high values of specific surface density of flag leaves (0.408-0.777 g/dm^2 and 0.509-0.596 g/dm^2), low values of length from flag leaf of the spike (11.5-15.9 cm and 7.7-12.6 cm), the optimal length of the last internode (15.3-19.0 cm and 15.4-18.5 cm), with small distances from the last node to the spike (28.2-33.4 cm and 23.6-29.8 cm). cm), high protein yields (85.6-91.8 g/m^2 and 62.8-69.4 g/m^2) per unit area.

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Variation of canopy temperature of the plant depending on water supply in wheat genotypes

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In the presented article, based on the results of research carried out in the "Plant Physiology" department and reference data, the canopy temperature of plants in different phases and genotypes depending on the water supply of wheat genotypes was studied. For the study, 7 local and introduced bread wheat genotypes with different morphological characteristics were taken and they were divided into 3 groups: early, medium, and late heading(spike). It was determined that the canopy temperature of the plant depends on the biological characteristics of the genotype as well as its water supply. In the article, canopy temperature under water scarcity was discussed, it was concluded that the leaves are protected from overheating as a result of high transpiration of the plant in the optimal irrigation regime, and it was shown that the course of normal physiological and biochemical processes leads to the formation of high productivity. Also, as a result of comparing the tolerance of genotypes with vertical and horizontal leaves to drought and heat stress, it was recommended to use the genotypes with vertical leaves in breeding and to give preference to genotypes that collect more biomass until the stress factors are aggravated.

***Keywords:** Wheat, genotype, drought, heat, tolerant, selection, productivity*

INTRODUCTION

Against the backdrop of ecological imbalance, environmental stress factors constantly limit crop production, creating a problem for sustainable wheat production in meeting the food demand of the population. Increasing tolerance to abiotic stresses is considered one of the main goals of increasing wheat production in the world. Among the abiotic factors, drought and heat stress or their combination is the main factor limiting productivity and causing disruption of many biochemical and physiological processes, including cell membranes (Tahmasebi et al., 2017).

Drought tolerance mechanisms are divided into 3 major groups: drought avoidance, resistance, and biochemical resistance of cells during water scarcity (Plomion et al., 2016). Heat tolerance in plants results in the accumulation of various metabolites, including antioxidants, osmotic regulators, and heat shock proteins (Fisher, Byerlee, 1990). Despite the fact that drought is a relatively effective process in nature due to the mechanism of action, heat stress occurs rapidly.

As a result of the increasing impact of global climate changes on the agricultural area (drought and heat stress), crop production is limited. Plants try to adapt to these stresses by showing morphological, physiological, biochemical and genetic reactions. In various studies, it is noted that grains are more sensitive in the reproductive stage of development, due to the effects of drought and heat, the grain filling weakens, as a result, the yield decreases.

Researchers indicate that the optimum temperature during flowering is 17.5ºC (Hasanuzzaman et al., 2013). When the air

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temperature is 1-2ºC higher than the optimum temperature for several days, the yield of wheat is significantly reduced (Siebert et al, 2014). At the same time, the importance of the difference between the temperature of the canopy and the air was noted in the studies, and it was shown that its optimal value is 7ºC. The difference between air and canopy temperatures is an indicator of heat resistance and is considered a valuable sign for breeding (Talaiet al., 2011; Neukam et al., 2016).

Quantitative values of drought and heat tolerance in the wheat plant reduce the success of such an approach by reducing the probability of growth and development of genotypes (Lipiec et al., 2013). Cereal producers mainly focus on yield management and its stability under stress conditions due to drought and heat tolerance (Gilliham et al., 2017). In addition to noting that plant phenotypic indicators are important for certain environmental conditions for the regulation of drought and heat stress, the identification of quality trait loci of many genes controlling them is highly appreciated (Tardieu et al., 2012). The effect of stress on crop production is influenced by many signs, including root properties of remobilization of assimilates accumulated in the total biomass, osmotic regulation, etc. affects and limits productivity (Iqbal et al., 2017).

Researchers note that heat and drought stress are often perceived together (Dreesen et al., 2012). The effect of heat and water stress reduces stomatal permeability in plants and weakens transpiration, resulting in an increase in leaf temperature (Król, 2013). Under the influence of stress factors, the assimilation surface of leaves decreases (Poorter et al., 2009). As a result of morphological changes, the cells in the leaves become smaller, which causes the stoma (pore) to be more closely spaced (Shahinnia et al., 2016). Various morphological, physiological and biochemical adaptations are formed as a response of plants to abiotic stresses (Huber, Bauerle, 2016). In order to avoid stress, the change of root architectonics in plants regulates the permeability of the stoma and the reduction of leaf area, the increase of leaf thickness, and finally the morphological and biochemical adaptation to stress by reducing the level of evapotranspiration (Goufo et al., 2017). The formation of a wax layer on the leaves of the plant is also a manifestation of the response to stress (Lee, Suh, 2013). Studies show that photosynthetic efficiency reduces the effects of heat and drought stress depending on the level of transpiration (Zandalinas et al., 2016). Due to the effect of high stress, the amount of chlorophyll decreases, thylakoid grains are broken and the transport of produced assimilations is disrupted (Kozłowska et al., 2007). In addition to managing yield under stress conditions and implementing irrigation to reduce harmful effects, it is desirable to create more resistant genotypes.

MATERIALS AND METHODS

Researches were carried out in the field of Absheron Subsidiary Experimental Farms of the Research Institute of Crop Husbandry. 7 local and introduced bread wheat genotypes with different morphophysiological characteristics were used for the study and they were divided into 3 groups as early (Gobustan, Giymatli 2/17 and Azamatli-95), medium (Gyrmyzy gul-1 and 12ndFAWWON N97) and late heading (4thFEFWSN N50 and Tale- 38) ones. The studied genotypes were also grouped by having different leaf architectures, i.e. vertical and horizontal to the planting surface. Researches were carried out in optimal irrigation, partial irrigation, drought, partial drought options depending on the water supply. In the optimal irrigation option, the field was irrigated twice in the vegetation year and the moisture content was 65-75%, in the partially irrigated option, irrigation was stopped after the first irrigation, the moisture content was 45-65%, and in the drought option, the moisture content was around 35-65% by creating an artificial drought condition, in the case of partial drought, the field was irrigated only in the second irrigation (at the end of April). During the study, the heading(spike) date of genotypes was recorded in all variants, and plant height was measured at the end of vegetation. Canopy temperature was measured with a portable infrared thermometer and measurements were taken twice during the day at 11^{00} and at 15^{00} - the hottest times of the day, and in three replicates at the beginning of the milk (26.05) and wax

ripeness (01.06) phases. The mass of the above-ground part and productivity were calculated according to the bundle taken from a 1 m^2 area (Dospekhov, 1985).

RESULTS AND DISCUSSION

In all studied genotypes, the canopy temperature was lower in the irrigation option compared to the other options in both phases, which is due to the large leaf surface and the opening of the stoma as a result of the optimum amount of water in the soil in the irrigation option. A normal water supply leads to more biomass formation per unit area, which increases the amount of evaporation, keeping the canopy cooler. The canopy temperature depends on the physiological state of plants. So, physiologically active, i.e., late-heading genotypes regulate the temperature better. In the partially irrigated option, the canopy temperature was high in the early heading genotypes, while it was relatively low in the medium and late heading genotypes. This is due to the fact that these medium- and late-heading genotypes are physiologically active.

In the second half of the day (at 15 o'clock), in addition to the regularity mentioned above, the temperature difference was less in varieties with a larger leaf surface, as the water balance in the plants decreased. In drought conditions, transpiration weakens due to high water scarcity in the soil, as a result of which the canopy temperature rises. High temperature in plants was observed mostly in genotypes with early heading and horizontal leaves.

In the partial drought option, the canopy temperature was lower compared to the drought option. This reduction was found to be relatively greater in mid- and late-heading and vertical-leaf genotypes. Plant temperature is also highly dependent on plant architecture. In genotypes with good architecture (vertical leaves - Azamatli-95, Gyrmyzy gul-1 and 12ndFAWWON N97), the sun rays penetrate deeper layers of the crop and as a result, the whole system is involved in the regulation of plant temperature, and in other types of genotypes (horizontal leaf-Giymatli 2/17, 4thFEFWSN N50 and Tale-38) the main function is performed by the 8th leaf, which prevents the sun's rays from penetrating more inside, as a result, the canopy temperature rises (Table).

As can be seen in the table (milk ripeness), the air temperature was 29ºC at 11^{00}, the highest canopy temperature in the optimal irrigation regime was in the Giymatli -2/17 variety with horizontal leaves (22.7ºC), the difference was 6.3ºC. The minimum value was 20.9ºC in genotype 4thFEFWSN № 50 with late-heading(spike) horizontal leaves, the difference compared to wheather was 8.1ºC. This happens due to the fact that the variety is physiologically active, at the same time, the remobilization of nutrients in the root has just started, and as a result of the high absorption power, there are sufficient assimilates in the root. This similar situation is also manifested in other early and late heading genotypes. In the partially irrigated variant, the minimum canopy temperature was 21.1, 21.5, and 21.4ºC in the late-heading 4thFEFWSN № 50, Gyrmyzy gul-1, and Tale-38 genotypes.

In the drought option, the lowest value of canopy temperature in this phase was 22.3ºC in Giymatli -2/17 from the early heading genotypes. This is characterized by high evaporation in the first half of the day as a result of relatively normal water balance in the plant.

In the measurements made at 15^{00} hours during the milk ripeness phase, the weather temperature reached 31ºC and the water reserves in the plants were exhausted, as a result, the value of the canopy temperature increased. In this phase, in optimal irrigation and partial irrigation options, the minimum value was in early-heading Gobustan and Azamatli-95 genotypes, and in late-heading genotypes, 4thFEFWSN №50. In the drought option, the minimum value was observed in the early-heading Azamatli-95, and in the late-heading genotypes, the Gyrmyzy gul-1 genotype.

At the beginning of the phase of wax ripeness, the plants have relatively lost their physiological activity, the water absorption force has decreased due to the transport of assimilates from the root, the weather temperature has increased, and at the same time, the drought has deepened. The regularity observed in the phase of milk ripeness has been shown relatively sharply in this phase as well.

Table. Canopy temperature depending on the water supply of different wheat genotypes

The name of the variety	Options	Heading date	Plant height, cm	26.05				01.06				Mass of bundle, g/m^2	Productivity, g/m^2
				11^{00} weather-29ºC		15^{00} weather-31ºC		11^{00} weather-30ºC		15^{00} weather-32ºC			
				Plant temperature	The difference	Plant temperature	The difference	Plant temperature	The difference	Plant temperature	The difference		
Gobustan	**I**	30.04	102	21.4	7.6	25.2	5.8	27,3	2.7	29.6	2.4	1707	598
	II	30.04	101	21.8	7.2	25.6	5.4	27.6	2.4	29.7	2.3	1690	565
	III	27.04	99.0	23.8	5.2	26.4	4.6	27,8	2.2	30.1	1.9	1560	447
	IV	27.04	100	23.2	5.8	26.1	4.9	27.6	2.4	30.0	2.0	1575	480
Giymatli 2/17	**I**	01.05	90.0	22.7	6.3	25.4	5.6	27,0	3.0	29.7	2.3	1770	525
	II	01.05	88.0	21.8	7.2	25.7	5.3	27.2	2.8	29.8	2.2	1775	490
	III	28.04	83.0	22.3	6.7	26.5	4.5	27.5	2.5	30.2	1.8	1277	320
	IV	28.04	84.0	23.3	5.7	26.2	4.8	27.3	2.7	30.3	1.7	1300	345
Gyrmyzy gul 1	**I**	08.05	80.0	21.2	7.8	24.8	6.2	26,0	4.0	29.1	2.9	1750	577
	II	07.05	80.0	21.5	7.5	25.0	6.0	26.3	3.7	29.4	2.6	1735	460
	III	06.05	76.0	23.0	6.0	25.8	5.2	27,3	2.7	29.9	2.1	1695	407
	IV	06.05	77.1	23.4	6.2	25.0	6.0	26.2	3.8	29.3	2.7	1720	480
Azamatli 95	**I**	30.04	105	21.5	7.5	25.2	5.8	26,1	3.9	29.5	2.5	1597	527
	II	30.04	103	22.0	7.0	25.5	5.5	27.5	2.5	29.6	2.4	1580	495
	III	28.04	95.0	22.9	6.1	25.9	5.1	27,7	2.3	30.2	1.8	1470	430
	IV	28.04	96.0	22.3	6.7	25.7	5.3	27.6	2.4	30.3	2.1	1490	475
Tale 38	**I**	10.05	108	21.1	7.9	24.5	6.5	26,1	3.9	29.3	2.7	1660	565
	II	10.05	105	21.4	7.6	25.1	5.9	26.3	3.7	29.4	2.6	1640	520
	III	08.05	92.0	23.1	5.9	26.0	5.0	26,6	3.4	29.7	2.3	1343	380
	IV	08.05	95.0	21.7	7.3	25.0	6.0	26.2	3.8	29.9	2.6	1475	480
12nd FAWWON N97	**I**	05.05	99.0	21.6	7.4	24.6	6.4	26,3	3.7	29.8	2.2	1413	405
	II	05.05	98.0	21.8	7.2	24.9	6.1	26.7	3.3	29.7	2.3	1380	370
	III	03.05	93.0	22.4	6.6	25.6	5.4	27,5	2.5	30.2	1.8	1240	310
	IV	03.05	94.0	22.2	6.8	25.0	6.0	27.1	2.9	29.9	2.1	1283	365
IthFEFWSN N50	**I**	10.05	108	20.9	8.1	24.4	6.6	24,9	3.1	29.2	2.8	1347	510
	II	10.05	106	21.1	7.9	24.8	6.2	24.3	5.7	29.4	2.6	1315	480
	III	08.05	103	21.8	7.2	26.0	5.0	25,4	4.6	29.9	2.1	1117	338
	IV	08.05	104	21.4	7.6	25.0	6.0	25.0	5.0	29.6	2.4	1145	470

Note: *I - optimal irrigation; II – partial irrigation; III-drought; IV- partial drought*

That is, the difference between the canopy temperature and the weather temperature decreased and was higher in the late-heading genotypes.

CONCLUSION

In conclusion, the amount of biomass collected in a single area plays a key role in the regulation of canopy temperature in wheat, along with its physiologically active state. The highest productivity is found in the early-heading or medium-heading genotypes that at the same time, form a high biomass. It is appropriate to choose these types of genotypes as the primary parental form in breeding and to include them in hybridization programs.

At the same time, it is appropriate to individually select the early-heading and medium-heading forms, which form more biomass due to the high coefficient of tillering,

and forms with vertical leaves for regular breeding programs.

Depending on the water supply, the decrease in productivity was observed in the early-heading genotypes under partial irrigation, and in the late-heading genotypes under drought. Taking this into account, it is appropriate to select the genotype according to the seasonal distribution of precipitation in rainfed regions.

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Productivity and quality indicators of bread wheat variety samples depending on irrigation

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The research was conducted in the Absheron area of the Republic of Azerbaijan, to identify the relationship between the grain yield and certain parameters of its technological quality-mass fraction and quality of raw gluten in grains and content of protein of winter bread wheat variety and samples. In the vegetation seasons of 2020 and 2021 year fourteen wheat varieties of different geographical origin were studied. Despite the contrast between the years of research in weather conditions, most of the studied variety samples were characterized by high grain quality. On average, over the years, the yield capacity of varieties varied from 333.0 g/m2 (Giymatli 2/17) to 704.0 g/m2 (Gyrmyzy gul 1). The highest protein and gluten content on average over two years of research was noted in the Nurlu-99 variety (confidence limit 13.9±0.3% and 30.5±0.37%), 31.6%, gluten quality (GQ) - 93.1 d.r. The correlation between yield and protein and gluten content in grain was r=-0.30 and r=-0.39 in 2020, r=-0.638*and r=-0.19 in 2021, respectively. In both years of research, statistically strong significant relations between protein and gluten content in grain were noted (r=0.35 in 2020, r=0.47 in 2021). A moderately conjugate relationship between the mass fraction of gluten and its quality was revealed in 2020 (r=0.51) and a positive weak relationship (r=0.28) was revealed in 2021. The conducted studies showed the possibility of obtaining high-quality grains of winter bread wheat in the conditions of Absheron. The results obtained allow us to recommend the use of these varieties as initial material in the breeding process for the creation of frost-resistant, productive, and good grain quality varieties of bread wheat.

Keywords: *Bread wheat, variety, yield, protein, gluten, gluten quality.*

INTRODUCTION

The climate of the Republic of Azerbaijan is temperate continental, not provided with enough moisture. Risky agricultural regions are associated with special climatic conditions expressed by the diversity of weather conditions in one or another district and region. This creates certain difficulties in the cultivation of winter bread wheat.

Wheat is of supreme importance among cereals mainly because of its grains, which comprise protein with exclusive physical and chemical attributes (Garg et al., 2021).

The growth period is one of the main problems in breeding. A complete growing season consists of the sum of two main intermediate periods: germination-earing and earing-ripening. Compared to the second main period - earing-ripening, the duration of the first period depends more on the biological characteristics of the variety than on the weather environment (Dilmurodovich, 2023).

It is necessary to take into account the relationship between quantitative and qualitative indicators. For example, it is known that the relationship between the mass of 1000 grains and the amount of protein is compensatory, therefore, both characteristics should be controlled in selection work for yield and product quality (Dilmurodovich et al., 2021; Dilmurodovich and Usmanovna, et al., 2021; Egamberdievna et.al., 2021).

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The amount of protein in wheat grain mainly depends on the climatic conditions of the growing year and also increases from west to east and from north to south (Egushova & Kondretenko**,** 2012). According to some investigations, the conditions of the year of cultivation have a significantly higher influence on the formation of the mass fraction of protein and gluten in grain (Sukhorukov et al., 2010; Fadeev et al., 2018).

The influence of various factors on the quality of gluten in wheat grain has been studied by many scientists Livia (Hajasa et al., 2018; Lie et al., 2018).

One of the most effective ways used to improve the quality of grain is the use of mineral fertilizers. Mineral fertilizers continuously increase the productivity of the soil, facilitate the use of moisture by the plant and ensure an increase in grain yield.

To improve grain quality with the application of mineral fertilizers, it is essential to take into account the regularity of protein formation in grain and its unchangeable characteristics. The meaning of grain quality should be approached from two main aspects- nutritional value and technological properties, important for the baking industry (Tomaz et al., 2021).

The nutritional value of wheat mainly depends on the chemical composition of the grain, which consists of proteins and amino acids. The main product made from wheat grain is bread. Well-baked bread acts as a unique catalyst, improves the digestion process, and accelerates the assimilation of other products by the body (Sozinov and Jemela, 1983).

Wheat is the source of approximately half of the food calories consumed worldwide and is rich in proteins (gluten), minerals (Cu, Mg, Zn, P, and Fe), vitamins (B-group and E), riboflavin, niacin, thiamine, and dietary fiber (Khalid et al., 2023). Wheat production and quality could be enhanced through the development of new and improved varieties that are able to produce a superior yield and perform better under various agro-climatic stresses and conditions (Hassan and Gul, 2006).

It has been proven in scientific studies that the duration of the plant growth period is determined by the natural variability of the variety and depends on the growing conditions (Dilmurodovich, 2023)

The high amount of gluten in bread wheat varieties mainly depends on the ecological conditions of the year, and the quality of gluten depends on the genotype as well as the year and cultivation conditions. The observed regularity did not manifest itself in the collection of protein.

The high amount of gluten in bread wheat varieties mainly depends on the environmental conditions of the year, and the quality of gluten depends on the genotype, year and growing conditions. The observed regularity did not appear in protein collection (Hasanova, 2015; Hasanova et al., 2016)

It is very important to clarify the influence of factors that have a high role in the formation of grain quality. Thus, the study of complex factors in field conditions and the application of these indicators by demonstrating them in farms will create an opportunity to obtain high-yield and high-quality grain products and create an abundance of grain in our Republic.

The purpose of the study was to identify winter bread wheat varieties that have the ability to form high-quality crops in the conditions of Azerbaijan. To achieve the goal, the amount of protein and gluten in grain and the quality of gluten, as well as the relationship of these indicators with productivity, were studied.

MATERIALS AND METHODS

In order to achieve the goal set in the research, in accordance with the methodology, 14 different bread wheat varieties and prospective samples selected from the wheat nurseries introduced from the International Centers as CIMMYT and ICARDA and obtained through hybridization in 2 variants (I-optimally irrigated and II-non-irrigated) were planted in field conditions at the Absheron Subsidiary Experimental Farms of the Research Institute of Crop Husbandry and technological quality indicators, grain yield were studied.

The amount of nitrogen in the grain was determined by the modified Keldal micromethod with the use of the KeltekTM FOSS device. The coefficient (Nx 5.7) was used to convert the value of nitrogen into protein (Pleshkov, 1976).

The vitreousness of the grain is determined by using the diafanoscope-DSZ-3 device, the amount of gluten by the method of weighing the dough obtained by separating the starch from the flour by hand washing, the quality of gluten (deformation index of gluten - GDI) was determined by using the

IDK-3M device and the sedimentation index was determined by recording the volume of swelling and sedimentation of high molecular weight protein particles in 2.0% glacial acetic acid (Guidelines for assessing the quality of grains and oilseeds, 1986).

The efficient use of water should be promoted by evaluating the grain yield based on the number of irrigations applied in the crop cycle so that under these conditions yields can be maintained) as well as industrial quality (Martínez et al. 2020).

RESULTS AND DISCUSSION

Grain yield and other structural elements of the investigated wheat varieties differ sharply from each other. This is due to the fact that in a well-developed wheat plant under optimum irrigation, the flow of carbohydrates to the grains is faster than that of protein substances, while in the non-irrigated variant, the flow of carbohydrates from the stem and leaves to the grain is weakened and the flow of protein substances is accelerated.

For this reason, the number and mass of grains in one spike and the weight of 1000 kernels increase and the amount of protein decreases in the optimal irrigation option. In the non-irrigated version, the number and mass of grains in one spike and the weight of 1000 kernels decrease and the amount of protein increases. These results also coincide with the procedures of other scientific studies (Huseynov et al., 2005; Strelnikov, 1971; Fadeeva et al., 2018).

Also, it was determined by some researchers that the amount of nitrogen fertilizers in cultivated soils is not enough for the formation of high protein substances in the grain of high-yielding wheat varieties (Huseynov, 2009; Konovalov, 1981).

The evaluation of the productivity in the studies conducted by us shows that the average maximum productivity in two years for the varieties was 704.0 g/m^2 in the optimal irrigation option, and 600 g/m^2 in the non-irrigated option. This level of productivity is quite high for the conditions of Azerbaijan in unfavorable years. Along with the productivity of the varieties, the technological quality indicators of the grain were also studied. The main quality indicators characterizing food grains are the amount of protein and gluten, as well as the quality of gluten.

According to State Standards requirements, the mass share of gluten in 1st class wheat grain should be (at least) 32%, the mass share of protein 14.5%, and 28% and 13.5% in 2nd class wheat grain, respectively. The conducted studies show that the average amount of protein in the grain of the vast majority of studied varieties was high (13.0-13.9%). The highest result (13.9±0.3% within the confidence) was recorded in the Nurlu 99 variety and was significantly different from other varieties. The maximum mass share of gluten in this variety was 31.6%, GDI -93.1 d.r. on average over the years (30.5±0.37% within the confidence) (Tables 1 and 2).

Table 1. Correlation coefficient between yield and grain quality indicators in bread wheat varieties and prospective samples in irrigation option (Absheron 2020-2021 average)

	Grain mass, g/m^2	**1000 grain weight, g**	**Grain moisture, %**	**Test weight, g/l**	**Protein content, %**	**Protein yield per hectare, kg/ha**	**Vitrousness, %**	**Gluten content, %**	**GDI, d.r.**	**Dry gluten content, g**
Grain mass, g/m^2	14									
1000 grain weight, g	-0.294									
Grain moisture, %	0.232	-0.295								
Test weight, g/l	0.376	-0.263	0.117							
Protein content, %	-0.638*	-0.129	-0.534*	-0.189						
Protein yield per hectare, kg/ha	0.976**	-0.367	0.119	0.371	-0.459					
Vitrouseness,%	0.428	-0.070	0.390	0.312	-0.428	0.394				
Gluten content,%	-0.193	0.125	-0.316	-0.723**	0.471	-0.092	-0.385			
GDI, d.r.	-0.069	0.436	-0.053	-0.344	-0.136	-0.110	-0.084	0.279		
Dry gluten content, g	-0.302	0.232	-0.527	-0.539*	0.564*	-0.188	-0.300	0.811**	0.061	
Sedimentation, ml	0.272	-0.312	0.300	0.134	-0.076	0.286	0.275	0.127	0.011	0.195

*. Correlation is significant at the 0.05 level. Optimum irrigation option. **. Correlation is significant at the 0.01 level.

Table 2. Correlation coefficient between yield and quality indicators of bread wheat varieties and prospective samples in non-irrigated option (Absheron 2020-2021 average)

	Grain mass, g/m²	**1000 grain weight, g**	**Grain moisture, %**	**Test weight, g/l**	**Protein content, %**	**Protein yield per hectare, kg/ha**	**Vitrousness, %**	**Gluten content, %, %**	**GDI, d.r.**	**Dry gluten content, g**
Grain mass, g/m²	1									
1000 grain weight, g	0.020	1								
Grain moisture, %	0.404	-0.651*	1							
Test weight, g/l	0.532	-0.457	0.341	1						
Protein content, %	-0.438	-0.212	0.008	-0.402	1					
Protein yield per hectare, kg/ha	0.980**	-0.017	0.425	0.499	-0.257	1				
Vitrouseness,%	0.443	-0.455	0.419	0.461	-0.155	0.447	1			
Gluten content,%	-0.157	0.179	-0.068	-0.415	0.403	-0.074	-0.047	1		
GDI, d.r.	-0.363	0.626*	-0.566*	-0.659*	0.069	-0.370	-0.318	0.317	1	
Dry gluten content, g	-0.218	0.230	-0.217	-0.362	0.412	-0.132	0.002	0.956**	0.324	1
Sedimentation, ml	0.382	-0.137	0.308	-0.072	0.491	0.506	0.011	0.204	-0.412	0.098

*. Corellation is significant at the 0.05 level. Optimum irrigation option. **. Corellation is significant at the 0.01 level.

A weak negative correlation between yield and grain protein content r=-0.30 and a significant negative correlation r=-0.39 between yield and gluten is observed in the varieties studied in 2020. It was also found that there is a relationship between the quality of protein and gluten and the mass fraction. In 2021, a weak negative correlation r=-0.19 was observed between yield and grain protein content, and a weak positive correlation r = 0.19 was observed between yield and gluten.

Based on the conducted research, it can be concluded that the weather conditions in the growing season of 2020 were not favorable for the collection of protein and gluten in winter wheat grain, and for this reason, the amount of protein and gluten in the grain of some varieties studied in 2020 was lower than in 2021.

When examining the relationship between the amount of protein and gluten in grain, it was found that there is a high positive correlation relationship (r=0.35 in 2020, r=0.47 in 2021) in both years. The correlation between wheat grain gluten and GDI was r=0.51, (moderate, significant value) in 2020 and weakly negative r=- 0.28 in 2021.

The above indicates that the agro-climatic conditions of our Republic are favorable for the breeding of high-yielding varieties and the production of high-quality grain. Such varieties include Gyrmyzy gul-1, Gobustan, Azamatli 95, Nurlu 99, and 12ndFAWWON N97, 4thFEFWSN N50 variety samples, and they have the potential to give 6.02 t/ha high-quality grain yield even in unfavorable weather conditions of the year.

CONCLUSION

The maximum amount of protein and gluten in the grain of the studied varieties was recorded in the 2020 research year. By results of the carried out correlation analysis the relationship between quality indicators and productivity of the winter wheat varieties was determined. It was also found that there is a relationship between protein content, gluten content, and gluten quality.

Thus, the high average value of protein was recorded in Nurlu 99 (13.9%), Gobustan (13.4%), Khazri (13.4%), Saratovskaya 29 (13.4%) varieties. - According to the mass share of gluten and the quality of gluten, the following varieties are Gobustan (38.0% and 90.3 d.r.), Khazri (34.8% and 112.0 d.r.), Giymatli 2/17 (32.8% and 99.6 d.r.), Dayirman (32.0% and 108.3 d.r.) was selected.

Gobustan, Azamatli 95, Gyrmyzy gul-1 and Nurlu 99 have the potential to give enough 3.1 t/ha of high-quality grain even in unfavorable weather conditions of the year. It is recommended to use the selected varieties and samples (Gobustan, Azamatli 95 and Nurlu 99, Gyrmyzy gul-1, 12ndFAWWONN97, 4thFEFWSN N50) in purposeful breeding works to improve the quality of winter wheat grain.

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Molecular evaluation of genetic diversity and genetic analysis of Azerbaijan sweet cherries (*Prunus avium* L.) using capillary electrophoresis with fluorescence-labeled SSR markers

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The sweet cherry (*Prunus avium* L.) is one of the world's most commercially important perennial crops and its improvement has been the focus of human effort for thousands of years. A collection of 74 accessions of Prunus avium L. from a research station, across regions in Azerbaijan representing the sweet cherry germplasm in the country, were examined to estimate genetic diversity and to identify genetic relationships among accessions using a set of 12 microsatellite (SSR) markers. Among the primers used, PaCITA 18, pchgmS 2, AK 193, PaCITA 14A, PaCITA 14B, and AK 200 are polymorphic and effective enough to differentiate between cherry genotypes. After calculating the genetic similarity index, a genetic link dendrogram was constructed using phylogenetic and structure analysis. We found the highest number of alleles at the AK 193 locus (12). The PIC value ranged from 0.0322 to 0.7753. Genetic diversity ranged from 0.0312 to 0.8016. We recorded the highest index of genetic diversity for the AK 123 locus. Thus, the results show that Azerbaijan germplasm has good potential for cherry genotypes for further breeding studies. The information obtained from SSR fingerprinting will be useful in optimizing the conservation of sweet cherry genetic resources present in the Azerbaijan region. However, this approach can be applied to optimize the conservation of local genetic resources of other stone fruit tree species.

Keywords: *Prunus avium* L., *SSR markers, genetic diversity, fluorescent capillary electrophoresis, DNA fingerprinting, structure analysis*

INTRODUCTION

Sweet Cherry (*Prunus avium* L.) belongs to the Rosaceae family and is a cross-pollinated, usually diploid (2n=2x=16) tree plant. The natural distribution area is the northern part of Western Eurasia and Africa. Sweet cherry probably originated from the area between the Caspian Sea and The Black Sea and was spread to Europe by birds (Webster, 1996; Blando & Oomah, 2019). The world production of sweet cherry is 2.200.000 tons and it is the fourth most productive after stone fruits such as plum, peach, and apricot. About 60% of the sweet cherry production is concentrated in seven countries (https://www.fao.org/statistics/en/). There are around 1,500 varieties of cherries in the world (https://www.agro.gov.az/az/bitkicilik/coxillikekmeler/gilas). 600 varieties of cherries were collected in France. These cultivars were also collected from the INRA cherry collection of the Prunus Genetic Resources Center near Bordeaux, France. 30% of them belong to the French national cherry collection (Barreneche, 2014). Other varieties are brought from many parts of the world and collected in France (Teribia et al., 2016). In the mid-twentieth century, the introduction of basic molecular technology, the establishment of phylogenetic relationships, the development of genetic markers, and the construction of linkage maps expanded our understanding of key aspects of *Prunu*s genetics (Baek et al., 2018). Sweet cherry collections from 19 European countries were genotyped by

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Barreneche and colleagues (2021) using 14 SSRs to assess genetic diversity parameters, estimate the levels of population structure, and identify excessive germplasm. In Japan, more than 200 traditional cherry cultivars are known (Kobayashi 1993), and they show diverse floral characteristics, including traits seldom found in the wild (Kato et al., 2014]. Ohta et al. (2005) characterized the genetic diversity of the flowering cherry *(Prunus* subgenus *aviufro*m) with 85 peaches using SSR markers. Genetic variability among flowering cherries was found to be higher than among peach and cherry cultivars (Ohta et al., 2005). Turkey also possesses a high amount of cherry production and genetic diversity in the cherry population. The genetic diversity of 78 local cherry varieties in Turkey using 4 AFLP and 6 SSR primers was examined (Gulen et al., 2010). According to the dendrogram created out of the UPGMA analysis, the varieties were divided into 18 different groups, and the highest degree of similarity was 70%. Based on the results, the authors concluded that local Turkish varieties have great genetic diversity (Gulen et al., 2010). Marti and colleagues (2012) performed a genetic analysis of a total of 114 cherry genotypes representing commercial and old cultivars from different parts of the world with 40 SNP markers and 7 SSR markers. The results obtained with both markers were compared. As a result, although the average number of alleles per locus, observed heterozygosity, expected heterozygosity, and number of polymorphic points were higher for SSR markers than for SNPs, cherry groups in the dendrogram constructed with both sets of markers showed similarity. Austrian sweet cherry germplasm accessions were genotyped using a harmonized set of 11 simple sequence repeat (SSR) markers optimized in two multiplexed PCR reactions. Thirty-eight distinct allelic profiles were identified (Schuller et al., 2021). Barreneche et al. (2021) genotyped subsets of sweet cherry collections from 19 European countries in Europe A total of 314 accessions, including landraces, early selections, and modern cultivars, were monitored, and 220 unique SSR genotypes were identified. All 14 loci were confirmed to be polymorphic, and a total of 137 alleles were detected with a mean of 9.8 alleles per locus.

The objective of the study. The cherry plant cultivated in Azerbaijan has gained fame in Europe and Asia for its high quality (Bekefi et al., 2014). The main reason for this is that nine of the eleven climate types known around the world are in Azerbaijan, which created a good opportunity to grow tropical fruit plants (avocado, mango, etc.) and other plants, especially stone fruit plants (cherry, apricot, plum, peach, etc.). Unfortunately, the genetic characterization of cherry cultivars has not been carried out in Azerbaijan. We investigate the genetic diversity of Azerbaijani cherry varieties, which represent the country's genetic stock, by using SSR markers to reveal the different and similar characteristics from other cherry-producing countries and determine the genetic relationship between them. The originality of this study lies in the fact that this is the first of its kind in Azerbaijan. To date, no research has been conducted to examine genetic variation at microsatellite loci in the local context and introduce cherry cultivars and forms in Azerbaijan. The results obtained with microsatellite markers can be useful in the field of conservation of genetic diversity of sweet cherries, as they will contribute to the reliable classification of samples and the creation of core collections consisting of genetically different cherry varieties. Equally importantly, the characterization of the cherry plant with molecular markers can be used as primary material in future research. Thus, the results of the molecular analysis allow for the categorization of genetically close genotypes and - avoid unnecessary combinations in the selection process in the future. In addition to traditional methods, molecular markers can help guide the search for new genotypes (new allelic diversity) to expand collections. The study's purposes are manifold, and presented below:

a) To determine the areals of varieties and wild forms of local and introduced cherry spread in different regions of Azerbaijan

b) Analyze its current state, identification based on molecular markers, determination of degrees of genetic kinship

c) Preparation of recommendations for effective use in food and breeding programs based on the obtained results

MATERIALS AND METHODS

Plant material. The 74 cherry genotypes used in the study were collected from research stations of 5 regions of Azerbaijan (Guba, Khachmaz,

Sheki, Agdash, and Tartar). Figure 1 shows the regions. Information on the studied genotypes is given in Table 1. Young leaves collected from these genotypes were immediately placed in zip-locked plastic bags containing silica gel for drying.

Fig. 1. Map showing collection sites of cherry (*Prunus avium* L.) sample used for SSR development and population structure analysis (1-Khachmaz; 2-Guba; 3-Aghdash; 4-Shaki; 5-Tartar).

DNA extraction stages. The extraction of nuclear DNA from sweet cherry genotypes was carried out in the biotechnology laboratory of the Genome and Stem Cell Center of Erciyes University in Kayseri, Türkeye. For DNA extraction, the leaf sample was taken from each genotype, crushed in liquid nitrogen, and pulverized. Genomic DNA was extracted from leaves using a modified cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle 1987; Hormaza 1999). 100 mg of the obtained plant powder was placed into a 2 ml tube. 1000 µl of 2xCTAB solution (2% CTAB, 0.1 M Tris HCl (pH = 8.0), 1.4 M NaCl, 20 mM EDTA) preheated to 65ºC and 1% β-mercaptoethanol (pH = 8.0) were added into the tube and mixed well until a homogeneous mass was formed in the Vortex. The obtained suspension was placed in a water bath (65ºC) for 20 minutes-, and inverted every 5 minutes.

Table 1. Information on the used sweet cherry sample

Samples	Sweet cherry-picking region	Origin	GPS
Samba	Guba SRB	Canada	3m-491m
Lapins	Guba SRB	Canada	3m-486m
0900-Ziraat	Guba SRB	Turkey	3m-489m
Jir Gilas	Guba, Zardabi village, y/a	Azerbaijan	4m-610m
Chagrayi Napoleon	Guba, Zardabi village, y/a	Russia	3m-487m
Sari Drogana	Guba SRB	Germany	3m-486m
Tezyetishen Kassini	Guba, Zardabi village, y/a	Germany	3m-331m
Ramon Oliva	Guba, Zardabi village, y/a	France	3m-487m
Regina	Guba, Zardabi village, y/a	Germany	3m-330m
Sweetheart	Guba, Zardabi village, y/a	Canada	3m-329m
Bianka Gozeli	Guba, Zardabi village, y/a	Germany	3m-416m
Sari Denissena	Guba, Zardabi village, y/a	Germany	3m-331m
Jir gilas-2	Guba, Zardabi village, y/a	Azerbaijan	3m-331m
Bigarro Burlat	Guba, Zardabi village, y/a	France	3m-426m
Agh Gilas	Guba, Zardabi village, y/a	Azerbaijan	3m-426m
Early Lory	Guba, Zardabi village, y/a	France	3m-426m
North Vander	Guba, Zardabi village, y/a	Germany	3m-330m
Gara Gilas	Guba, Zardabi village, y/a	Azerbaijan	3m-423m
May Gilasy	Guba, Zardabi village, y/a	Azerbaijan	3m-430m
Krim	Guba, Zardabi village, y/a	Ukraine	3m-493m
Gara Napoleon	Guba, Zardabi village, y/a	Russia	3m-485m
Frans Iosif	Guba, Zardabi village, y/a	Czechia	3m-487m
Gara jir gilas	Sheki Supporting Point	Azerbaijan	3m-423m
Murebbe agh gilas	Sheki Supporting Point	Azerbaijan	3m-329m
Agh jir gilas	Sheki Supporting Point	Azerbaijan	4m-328m
Gara Shabalidi	Sheki Supporting Point	Azerbaijan	3m-334m
Gara Gilas	Sheki Supporting Point	Azerbaijan	3m-333m
Ala Gilas	Sheki Supporting Point	Azerbaijan	3m-339m

Continued Table 1

Samples	Sweet cherry-picking region	Origin	GPS
Jir gilas kesikli	Sheki Supporting Point	Azerbaijan	3m-331m
Okuzureyi agh	Sheki Supporting Point	Azerbaijan	4m-344m
Okuzureyi Gara	Sheki Supporting Point	Azerbaijan	3m-338m
Gizil Gilas	Sheki Supporting Point	Azerbaijan	3m-339m
Kahraba Gilas	Sheki Supporting Point	Azerbaijan	3m-340m
Alij Gilas	Sheki Supporting Point	Azerbaijan	3m-346m
Agh Gilas	Sheki Supporting Point	Azerbaijan	3m-377m
Krim	Sheki, Cheshmali village, y/a	Ukraine	3m-313m
Napoleon- Sheki	Sheki, Cheshmali village, y/a	Russia	3m-313m
Balli Gilas	Sheki, Cheshmali village, y/a	Azerbaijan	3m-314m
Jir gilas aji	Sheki, Cheshmali village, y/a	Azerbaijan	3m-312m
Jir gilas-2	Sheki, Cheshmali village, y/a	Azerbaijan	3m-312m
Mayovka girmizi	Sheki, Cheshmali village, y/a	Ukraine	3m-318m
Dum agh Gilas	Sheki, Cheshmali village, y/a	Azerbaijan	3m-318m
Albali gilas yumru	Sheki, Cheshmali village, y/a	Azerbaijan	3m-314m
Mayovka chil-chil	Sheki, Cheshmali village, y/a	Ukraine	3m-313m
Gara Mayovka	Sheki, Cheshmali village, y/a	Ukraine	3m-322m
Sari Gilas	Sheki, Cheshmali village, y/a	Azerbaijan	3m-322m
Albali gilas agh	Sheki, Cheshmali village, y/a	Azerbaijan	3m-318m
Sari uzun Gilas	Sheki, Cheshmali village, y/a	Azerbaijan	4m-322m
Guzugoren	Sheki, Cheshmali village, y/a	Azerbaijan	4m-322m
Gara Okuzureyi	Tartar, Alasgarli village, y/a	Azerbaijan	3m-197m
Zoghali	Tartar, Alasgarli village, y/a	Azerbaijan	4m-200m
Chal Krim	Tartar, Alasgarli village, y/a	Ukraine	3m-196m
Gejyetishen okuzureyi	Tartar, Alasgarli village, y/a	Azerbaijan	3m-197m
Yabani Gilas	Tartar, Alasgarli village, y/a	Azerbaijan	3m-199m
Napoleon	Tartar, Alasgarli village, y/a	Russia	3m-210m
Shampan Gilas	Tartar, Alasgarli village, y/a	Russia	3m-197m
Agh Krim	Tartar, Alasgarli village, y/a	Ukraine	3m-197m
May gilasi agh	Aghdash, Yukhari Gasil village,y/a	Azerbaijan	3m-43m
Agh Gilas	Aghdash, Yukhari Gasil village, y/a	Azerbaijan	3m-44m
Ala Gilas	Aghdash, Yukhari Gasil village, y/a	Azerbaijan	3m-43m
Gara okuzureyi	Aghdash, Yukhari Gasil village, y/a	Azerbaijan	3m-43m
Tezyetishen Krim	Khachmaz, Gochagli village, y/a	Ukraine	3m-151m
Napoleon	Khachmaz, Gochagli village, y/a	Russia	3m-142m
Krim gejyetishen	Khachmaz, Gochagli village, y/a	Ukraine	3m-141m
Agh Gilas	Khachmaz, Gochagli village, y/a	Azerbaijan	3m-140m
Xrustal	Khachmaz, Gochagli village, y/a	Azerbaijan	3m-141m
Ramon Oliva	Khachmaz, Gochagli village, y/a	France	3m-143m
Tezyetishen Krim	Khachmaz, Gochagli village, y/a	Ukraine	3m-134m
Erken Krasnodar	Khachmaz, Gochagli village, y/a	Russia	3m-137m
Jir Gilas	Khachmaz, Gochagli village, y/a	Azerbaijan	3m-140m
Alyanag	Khachmaz, Gochagli village, y/a	Azerbaijan	3m-142m
En gecyetishen Krim	Khachmaz, Gochagli village, y/a	Ukraine	3m-141m
Gara Krimson	Khachmaz, Gochagli village, y/a	Ukraine	3m-140m
Regina	Khachmaz, Gochagli village, y/a	Germany	3m-140m

* SRB (Scientific Research base); y/a (yard area)

After cooling at room temperature for 5 min, 700 µl of chloroform: isoamyl alcohol (24: 1) (CHIA) was added to the suspension and kept on ice for 30 minutes after inversion 20-25 times. In this case, all the components containing protein and phenol are dissolved, except for DNA and RNA. The mixture was centrifuged at room temperature at 14000 rpm for 5 min and the supernatant was transferred to another 2 ml tube. The stage was repeated by adding CHIA again. To precipitate DNA, 800-850 µl of cold isopropanol was added to the supernatant, and the tube was

sealed with paraffin, carefully mixed, and kept at -20^{0}C for 1 day. Samples were kept at -20^{0}C and centrifuged at 14000 rpm for 1 minute. After transferring the supernatant to a new tube, it was centrifuged twice by adding a cold washing solution (76% ethanol and 10 mM ammonium acetate). It was kept at room temperature for 30 minutes for drying and then 100 µl of TE (ph: 8) was added. This DNA solution was used as a reserve. DNA was quantified using a spectrophotometer, then 1 µl of RNAse was added and stored at 37°C for 30 min.

SSR primers and polymerase chain reaction (PCR) amplification. Initially, 157 SSR primers were available in the laboratory and used for apricot studies. Initial screening of the 157 SSR primers on eight cherries was performed with PCR reactions in 20 µl volumes containing 2 µl 10 x PCR Buffer (100 mM Tris-HCl pH 8.0 at 25ºC, 500 mM KCl, 0.8% (v/v) Nonidet), 2 µl dNTP, 2 µl $MgCl_2$, 0.25 µl forward primer (F), 0.25 µl reverse primer (R), 2 µl genomic DNA, 0.2 µl Taq polymerase and 11.3 µl ddH_20. The PCR amplification procedure was conducted at 94ºC for 3 min, followed by 35 cycles of 94ºC for 45 s, 55ºC for 1 min, and then 72ºC for 1 min, and a final extension step at 72ºC for 5 min. PCR products were separated by 3% agarose gel 1 x TBE buffer at 180 V for 2 h. The bands in the gel were stained with the ethidium bromide solution and examined under ultraviolet light. The SSRs producing polymorphic, robust, bright bands for the 8 cherry genotypes were selected according to the agarose gel view.

Among the 157 SSRs, 12 primer pairs producing clear, simple, and repeatable bands were selected to analyze the 74 cultivars (Table 2).

Allele sizing, cluster, and structure analysis. Fluorescently labeled SSR genotyping and PCR were performed for the 12 SSRs according to the method described by Schuelke (2000) for allele sizing and characterization of the loci that appeared polymorphic in the initial screening on agarose gels. PCR reactions were performed including M13 primer fluorescently labeled with 6-FAM, NED, PET, or VIC amplifying the same 74 cherries.

Table 2. Primers used in simple sequence repeat (SSR) analysis of cherry cultivars with fluorescent capillary electrophoresis

Locus	Forward and reverse primer sequences (5′-3′)	T_m, ºC
SSR PaCITA 18	F:CACGACGTTGTAAAACGACGCCGGTAGCTTTCGATTTCAAAC R:CCTAGGCTTCTATTCCCCTCACGAC	55
SSR pchcms2	F:CACGACGTTGTAAAACGACAGGGTCGTCTCTTTGAC R:CTTCGTTTCAAG91GCCTG	53
SSR AK 69	F:TGAAACTGAGGACGATGACG R:CGTCTTCCGGATTTGCTTTA	50
SSR AK 123	F:TGACATGCGCACTCTTCTCT R:CAGTTGGTAGGCCCTGGTAA	50
SSR PaCITA 10	F:CACGACGTTGTAAAACGACGGTGAGGTCTGTGCTGAATATGCCA R:CGATTAAAGAAATAAGAAAAAGAGC	55
SSR PaCITA 12	F:CACGACGTTGTAAAACGAGACACCCCAACCCACCCATCATGT R:GGTSTTGGAAATGTGGAAAGAAATG	56
SSR PaCITA 14A	F:CACGACGTTGTAAAACGACCCTTCAATGCTGGCATGGTTTCTTC R:GGAGAGAGGGTAGCTAGGGGGAGG	55
SSR PaCITA 14B	F:CACGACGTTGTAAAACGACCCTTCAATGGTGGCATGGTTTCTTC R:GGAGAGAGGGTAGCTAGGGGGAGG	55
SSR AK 193	F:GCAAATCAGCTAGTGAAAGA R:TACCACTTTACGATGTGTCGTT	53
SSR AK 200	F:CCAGTAGATTGGGTGCTACT R:CTACGTCCAAGAACAAGATT	53
SSR pchgms2	F:CACGACGTTGTAAAACGACGTCAATGAGTTCAGTGTCTACACTC R:AATCATAACATCATTCAGCCACTGC	53
SSR AK 178	F:GCACCAACTGTTCCATTTGA R:TGTCTTGATGTGAACCATGC	55

An electronic version of a test table was made, and the machine table was generated automatically. A mixture of 980 μL HIDI and 20 μL LIZ 600 was placed in a 96-well reaction plate with a continuous pipette, with each well having a volume of 10 μL. The well plate was sealed with sealing plate film, and placed in a flat plate centrifuge. In the PCR instrument, the denaturation process was conducted at 94^0 C for 5 min, without heating the hot cover, and at the end of the procedure, the 96-well plate was placed immediately on iced water. Once cooled, the well plate was placed in a flat plate centrifuge and exposed to an RCF. A 1 μL aliquot was loaded into an ABI 3500 capillary electrophoresis instrument (Applied Biosystems, Foster City, CA, USA) (Chao et al., 2007).

A genetic similarity matrix based on the proportion of shared alleles was generated and the expected heterozygosity (He), observed heterozygosity (Ho), and polymorphism information content (PIC) were calculated using the PowerMarker V3.25 software (Liu and Muse, 2005). The neighbor-joining (NJ) method used a matrix using the MEGA6 (Tamura et al., 2007) and PowerMarker V3.25 software programs. Both were used to identify the relationship between species.

The genetic structure in different of the 74 cherry cultivars was analyzed using 12 SSR primer pairs by model-based Software Structure v.2.3.4. by Pritchard et al. (2000). The most likely number of genetic clusters (k) in the Azerbaijan cherry population was also estimated by calculating the Δk values in line with Evanno et al. (2005) using STRUCTURE HARVESTER (Earl and vonHoldt 2012).

RESULTS AND DISCUSSION

As research material, 31 local, 34 introduced and 9 wild forms of 74 samples of sweet cherry discovered as a result of scientific expeditions in the Guba, Khachmaz, Sheki, Agdash, and Tartar regions of Azerbaijan, belonging to separate farms, backyards, scientific research and experimental bases were used, and their areals were determined using GPS technology (Table 1). Twelve SSR loci which could be scored with confidence for eight cherries were further used for the fingerprinting of the 74 cherries. The PCR products of 12 SSR loci were obtained, and their fragments were analyzed. The alleles were scored, and the SSR characteristic values, namely the number of alleles (n), He, Ho, and PIC, for the loci were calculated. Twelve microsatellite primers (SSR) were used to characterize sweet cherry (*Prunus avium* L.) samples. The genetic parameters for each locus - allele number (n), allele frequency (%), expected heterozygosity (He), observed heterozygosity (Ho), and detection probability (PI) described in the paper, were determined by the IDENTITY 1.0 (Wagner and Sefc 1999) program and calculated using the Paetkau vd. Method (1995). After calculating the genetic similarity index, a genetic link dendrogram was constructed using the UPGMA method (Sneath and Sokal 1973). Based on the genetic parameters obtained, 6 alleles were expected at the PaCITA 18 and AK 200 loci, while 5 alleles were detected at the pchcmS 2 and pchgmS 2 loci. The largest number of alleles was found in the AK 193 locus (12). A total of 54 alleles were found in 12 SSR loci. The PIC value ranged from 0.0322 to 0.7753. Genetic diversity ranged from 0.0312 to 0.8016. The allele frequency ranged from 0.2929 to 0.9841. The highest index was recorded for the AK 123 locus. Thus, all genetic parameters are listed in Table 3.

Structure and phylogenetic analysis. Population structure analysis by STRUCTURE software (Pritchard et al. 2000) revealed four sub-groups in Azerbaijan cherries for all the group number (k) tested from two to seven. For an illustration of four sub-groups in cherry in Azerbaijan, the graphs of estimated sub-populations are depicted in Figure 2 for k=4. The sup-population number was also estimated by calculating Δ k values as described by Evanno et al. (2005). As a result, the maximum Δ k value was reached at k=4.

Consequently, the most likely number of subpopulations in cherry samples collected in Azerbaijan is four. The four sub-groups in Cherry were named P1, P2, P3, and P4. Populations are shown in Figure 3B. A UPGMA cluster based on the shared allele distance was generated. The dendrogram is shown in Figure 3A. The cherry samples showed considerable spread in the dendrogram, with a low tendency to cluster by geographical origin. Thirty-three accessions were assigned to the P1 subpopulation, and most of the genotypes (16) assigned to P1 were clustered in the Shaki region of the UPGMA tree.

Table 3. Sequences of the 12 microsatellites (SSR) primers used for the characterization of 74 cherries (*Prunus avium* L.) accessions including the number of alleles per locus, allele frequency, expected heterozygosity, observed heterozygosity, genetic diversity, and polymorphism information content (PIC)

Primers and source plant	Source	Allele frequency	Number of alleles	Gene diversity	Heterozygous	PIC
PaCITA 18	Lopes et al. (2002)	0.4923	6	0.6347	0.8769	0.5721
pchcms2	Sosinski et al., (2000)	0.4727	5	0.5893	0.1636	0.5038
AK69	Kose et al. (2017)	0.9833	2	0.0328	0	0.0322
AK123	Kose et al. (2017)	0.9841	2	0.0312	0	0.0308
PaCITA10	Lopes et al. (2002)	0.7576	4	0.4008	0	0.3716
PaCITA12	Lopes et al. (2002)	0.9583	4	0.0807	0.0556	0.0791
PaCITA14A	Lopes et al. (2002)	0.4931	3	0.5136	0.9861	0.3952
PaCITA14B	Lopes et al. (2002)	0.5071	2	0.4999	0.9857	0.3749
AK193	Kose et al. (2017)	0.2929	12	0.8016	0.7857	0.7753
AK200	Kose et al. (2017)	0.4914	6	0.5489	0.9483	0.4473
pchgms2	Sosinski et al., (2000)	0.5069	5	0.5262	0.9167	0.4147
AK178	Kose et al. (2017)	0.9514	3	0.0935	0.0139	0.0909
Total		7.8911	54	5.2214	5.7325	4.0879
Mean		0.6576	4.5	0.3961	0.4777	0.3407

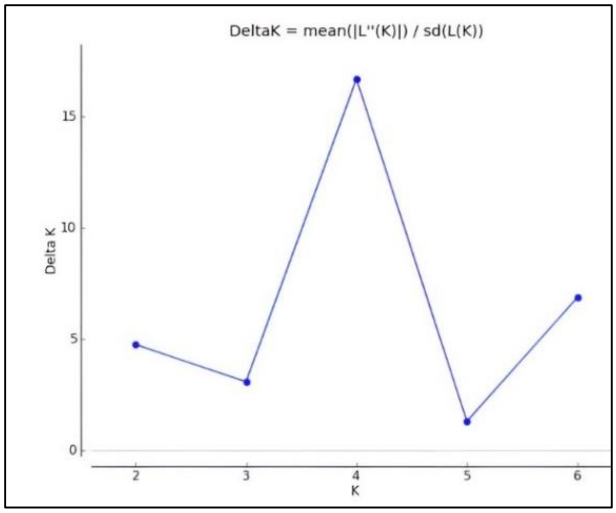

Fig. 2. Estimation of Dk in the 74 cherry accessions (K is the number of populations)

The eighteen samples of the P2 subpopulation were suggested by the structure analysis clustered. Considering P2, again like the P1 subpopulation, mostly the samples were not clustered together according to their geographic origin or their collection sites. In the sixteen genotypes of the P3 population, in brief, both the UPGMA tree and the structure analysis put together some geographically close samples but also placed some other geographically distant areas samples into the same group. The seven samples of the P4 subpopulation suggested by the structure analysis also clustered together on the lower part of the UPGMA tree. Five of the 7 samples of the P4 subpopulation were collected from Tartar. However, the other two samples of the P4 population Aghdash (1 sample), and Khachmaz (1 sample) were collected from distant locations.

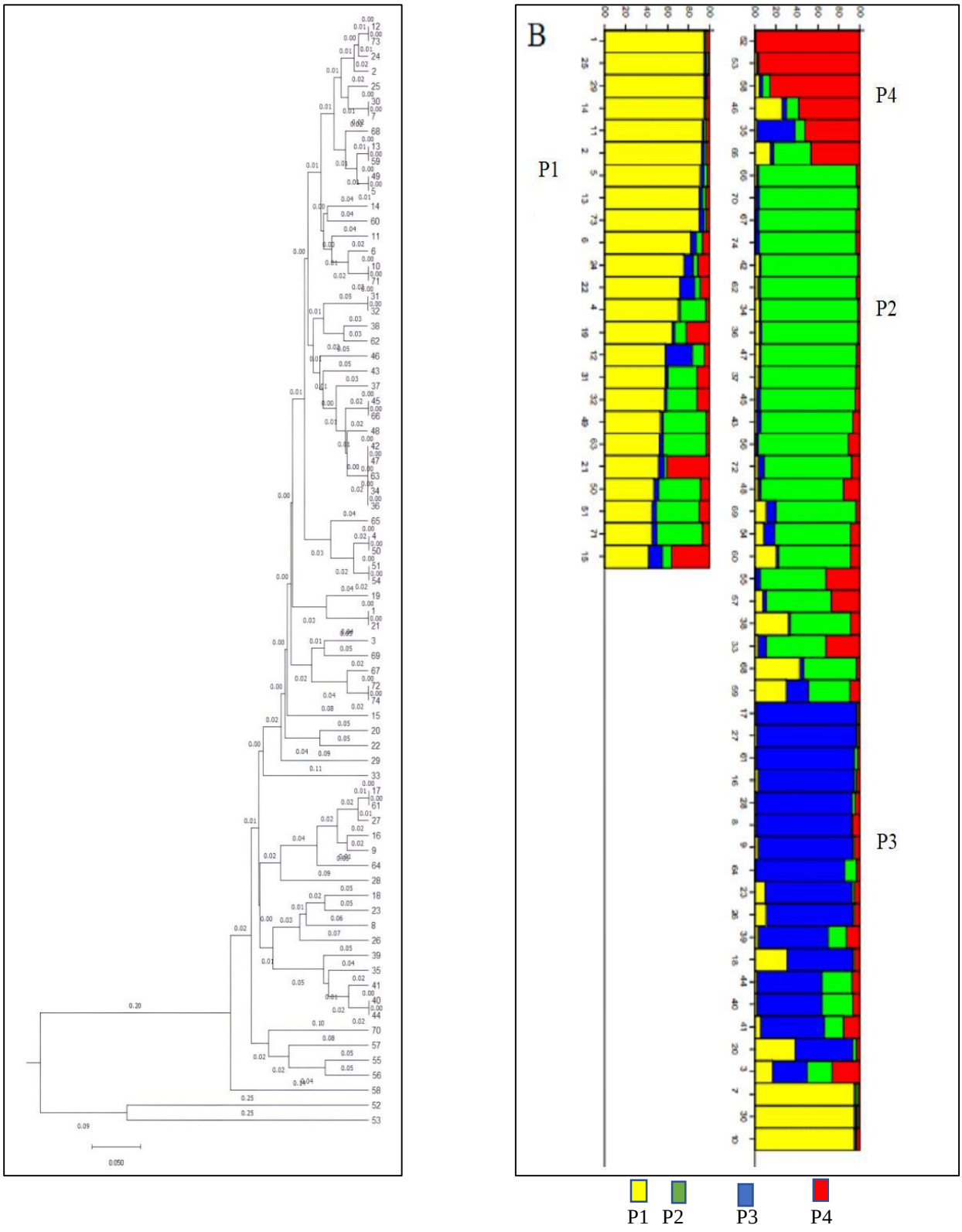

Fig. 3. UPGMA clusters (A), and Population STRUCTURE analysis (B) of 74 cherry genotypes based on twelve SSR loci.

In the last decade, the availability of reliable molecular markers is of great importance for plant breeding. In this study, genetic variation in sweet cherry cultivars of Azerbaijan has been assessed using SSR markers. The results are compared with the recently published genetic analysis of sweet cherry: Krmpot et al. (2020) investigated the genetic variability of 14 genotypes of sweet cherries using 26 SSR markers. Eight autochthonous sweet cherry genotypes from four different locations and six virus-free reference cherry varieties were included in the study. The average genetic distance between them was 0.43. The number of alleles per locus ranged from two to eight. The minimum number of two alleles of polymorphic loci showed EMPa003 and EMPa002, while the highest number of eight loci alleles had PceGA34 and UDP97-402 (Tanja Krmpot et al., 2020). Farsad and Ashari characterized 23 important Iranian sweet cherries (Prunus avium) cultivars collected from different provinces of Iran and 1 foreign cultivar, which was used as control, considered for breeding programs by using 21 microsatellite markers. Out of 21 SSR markers, 16 were polymorphic, producing 177 alleles that varied from 4 to 16 alleles (9.35 on average) with a mean heterozygosity value of 0.82 that produced successful amplifications and revealed DNA polymorphisms. Allele size varied from 95 to 290 bp (Farsad and Esna-Ashari, 2016).

Cherry breeding programs and the development of superior cherry cultivars are important needs in Azerbaijan and other cherry-rich countries.

The findings of this analysis must be considered in light of a few limitations. The low number of SSRs used in this study can be a drawback and the reason why samples are not separated according to their geographic origin on the Structure analysis and UPGMA dendrogram. Moreover, this approach may also be due to cross-regional migration or breeding and cultivation in different regions, indicating the complex nature of the history of sweet cherry domestication.

However, it is worth mentioning that for the old genetic studies on wild cherry, the result of the structural analysis using the data obtained with 8 SSR primers of 278 cultivars from 11 populations of wild cherry in Italy was found to be K=11. It has been reported that there is no specific geographical structure in the populations (Rogatis et al., 2012). The K value was determined as 2 in the structural analysis made with the data obtained as a result of the analysis of 131 wild cherry accessions sampled from 5 populations in northern, north-west and central Spain with 9 SSR loci (Fernandez-Cruz et al., 2014). The K value was found to be 5 as a result of the structural analysis performed with 11 SSR loci in 93 samples from 5 populations of wild cherry in Greece (Ganopoulos et al., 2011).

The genetic relatedness reflects the difference in genetic background between cultivars, so it is possible to breed elite cultivars by selecting genetically distant cultivars as hybrid parents. Genetic relatedness and genetic distance enable the development of optimal cultivars and rootstocks into superior cultivars.

In conclusion, the genetic structure and polymorphic SSR loci for cherry varieties cultivated in Azerbaijan have not been reported to date. In our study, the size and structure of genetic diversity in 74 sweet cherry trees sampled from 4 subpopulations of Guba, Khachmaz, Shaki, Tartar, and Aghdash regions were analyzed using SSR markers. As a result of the analyses and assessments, important information was obtained on the studied populations of sweet cherries.

CONCLUSION

This study represents an important step in the improvement protection and conservation of the cherry tree, which is gradually declining in the forests of Azerbaijan due to various factors. The study of the cherry gene pool using molecular markers allows both scientific and practical organization of their effective use. Thus, as a result of our studies, it was confirmed that microsatellite loci are a powerful tool for determining genetic differences between cherry genotypes. Geneticists and breeders can use this information, which reflects the degree of relatedness of the samples, to select varieties to cross for genetic studies. Despite its potential benefits, the study's authors have several recommendations for future studies. Thus, in addition to these populations, it is recommended to identify different populations belonging to the sweet cherry tree in Azerbaijan. In addition, the results obtained are recommended to geneticists

and breeders in the selection of samples to be crossed for genetic studies. Selected high-yielding, large-fruited, good-looking sweet cherry genotypes that meet market demand (Samba, Ziraat 0900, Regina, Ramon Oliva, Gara Okuzureyi, Agh gilas, Chahrayi Napoleon) can be used in breeding programs to create new varieties. Nuclear microsatellites AK 193, PaCITA 10, and PaCITA 18 are recommended for the characterization of genetic diversity with SSR primers. The early-ripening Chal Crimea and late-ripening Okuzureyi genotypes with rare alleles can be used as initial material for the improvement of new varieties.

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The III Edition of the "Red Book" of the Republic of Azerbaijan in solving the issue of biodiversity protection

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Due to the complexity of the natural climatic conditions, and geological relief structure, the Caucasus region, including Azerbaijan, is one of the main rich centers of biodiversity in the world. The impact of global climate change, expanding anthropogenic factors, and the fact that Armenia has exposed Azerbaijan's territories to environmental terrorism in violation of international law and conventions by 2020 have severely harmed the region's unique biodiversity. Red lists and the "Red Book" of the Republic of Azerbaijan are drawn up in accordance with the international system to save elements of biological diversity that are in danger of disappearing for one reason or another. In 2023, with the joint efforts of the Ministry of Ecology and Natural Resources and the Azerbaijan National Academy of Sciences, the third edition of the "Red Book" was published. With the liberation of Karabakh from occupation and the restoration of Azerbaijan's sovereignty, a historical opportunity has arisen for the preservation and efficient use of the biological diversity of the region, and the publications have also covered the liberated territories. The current edition of the "Red Book" includes 241 rare and endangered animals, 460 plants and fungal species. The third edition of the "Red Book" is an important contribution of the Azerbaijan National Academy of Sciences and the Ministry of Ecology and Natural Resources of the Republic of Azerbaijan, scientific institutions, scientists, and specialists to the "Year of Heydar Aliyev".

Keywords: *"Red book", flora, fauna, endangered and rare species*

Due to the variety and complexity of the natural climatic conditions and geological relief structure, the Republic of Azerbaijan occupies one of the most advanced places in the world in terms of the richness of biodiversity. Studies revealed that the country is one of the centers of the initial formation and endemism of a number of flora and fauna species [Flora of Azerbaijan; Animal World of Azerbaijan. Vertebrates, 2004; Asgarov, 2016; Ibadullayeva, Gahramanova, 2016; Mammadov et al., 2016; Talibov et al., 2021]. However, the loss of natural environments due to anthropogenic landscapes, including the expansion of urbanization and agricultural areas, the intensification of global climate changes, the strengthening of fragmentation and other negative stress factors, has led to the disappearance of some plant and animal species, and many are facing the threat of extinction. Another serious threat to the biodiversity of Azerbaijan in the last 30 years was the occupation of 20 percent of the territory of the republic that remained under occupation until 2020 and subjected to environmental terrorism by Armenia.

As a result of the damage to natural landscapes and forest cover by the invaders and the use of prohibited weapons during military operations, contrary to a number of international conventions that the Republic of Azerbaijan joined (1993 - UN Convention on Biological Diversity: https://www.cbd.int; 1993 - UNESCO Convention on the Protection of the World Cultural and Natural

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Heritage: http://whc.unesco.org/en/convention; 1998 - The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) https://www.cites.org; 1998 - The UNECE Convention on Access to Information, Public Participation in Decision-making and Access to Justice in Environmental Matters (Aarhus Convention), 1999- Convention on the Conservation of European Wildlife and Natural Habitats. (Bern Convention): https://www.coe.int/en/web/bern-convention; 2005- The Cartagena Protocol on Biosafety to the Convention on Biological Diversity; 2000 -The Convention on Wetlands of International Importance, especially as Waterfowl Habitat (Ramsar Convention): https://www.ramsar.org; 2011 - European Landscape Convention: https://www.coe.int/en/web/landscape), the UN Sustainable Development Goals, and other relevant international documents and legislative acts, the unique biodiversity of the region was seriously damaged. It is encouraging that Azerbaijan has already fully restored its sovereignty over all of Karabakh's territories and that opportunities for studying the territories and protecting biodiversity have increased.

As previously stated, a combination of these or other stressors, primarily humans' exploitative attitude toward nature, leads to a decrease in species diversity, which ensures the continuation of living life on Earth, as well as the extinction of many valuable plant, fungal, and animal taxa. Realizing the gravity of the situation, progressive humanity, particularly, the scientific circles raised the alarm from the middle of the last century and managed to make the appropriate authorities respond to this dangerous process. The world community has adopted international environmental conventions, including biological diversity, and other important documents, and all possible measures have been taken to ensure their implementation. Red lists of endangered species at the international, regional, and national levels are prepared and regularly updated, and the necessary information on these lists is published in the form of official "red books" of countries and certain regions.

An appropriate system was established by the International Union for Conservation of Nature [IUCN] in 1989 to compile red and pink lists of endangered species, to define categories and criteria for assessment, and to classify species at high risk. The Red Survey books have been used since 1994 to specify the categories and criteria of endangered species, and the lists, as well as the categories and criteria, have been updated and modified several times for over 30 years.

The Republic of Azerbaijan was not left out of the process. Preparation and publication of red lists with the support of the state, measures taken for the protection of rare and endangered species have always been in the spotlight. The most important of these measures is the regular preparation and publication of the "Red Book" of the Republic of Azerbaijan, which is an official state document on the functional status of rare and endangered species of wild animals, plants, and fungi in the territory of the republic. Like all over the world, the red list of fauna and flora includes the alpha, beta, and gamma early warning systems at diversity, species, and ecosystem levels, and also determines the direction of conservation activities.

Species of fauna and flora are included in the "Red Book" of the Republic of Azerbaijan in 2 categories: endangered and rare species.

The first category includes species whose number and range have significantly decreased and reached a crisis level as a result of the influence of some negative factors (loss and destruction of habitats). Species that tend to decrease in number and are found in small areas belong to rare species.

Animal and plant species are considered to be rare when there is no proper information about their number and reserves in nature and certain difficulties exist in organizing their protection.

Since the beginning of the 1970s, due to the purposeful policy and continuous activity of the National Leader of the Azerbaijani people, Heydar Aliyev, a large amount of work has been carried out for the restoration, reintroduction, and expansion of the population areas of a number of plant and animal species that are in danger of extinction in the country.

In the Azerbaijan SSR, by this great statesman’s will, 8 very important laws on ecology, environmental protection, and efficient use of natural resources, 32 decisions of the Central Committee of the Communist Party of Azerbaijan and the Council of Ministers were adopted in 1969-1982. It is also important that the first government

decision to establish the country's "Red Book" for the protection of the rich gene fund of the flora and fauna of the Republic of Azerbaijan coincided with the period (1977) when the Great Leader led Azerbaijan.

It took 12 years to implement this decision. The "Red Book" covering the territory of the country was published for the first time during the Soviet period - in 1989 [The "Red Book" of the Azerbaijan SSR, 1989]. The second edition of the "Red Book" was prepared much later - in 2013 under the leadership of a great scientist, public and political figure, academician Jalal Aliyev, and was published in 2 volumes [The "Red Book" of the Republic of Azerbaijan. Fauna, 2013; "Red Book" of the Republic of Azerbaijan. Flora, 2013]. In 2010, the "Red Book" of the Nakhchivan Autonomous Republic was also published [Talibov, Ibrahimov, 2010].

140 rare and endangered plant species are included in the first edition of the "Red Book" of the Republic of Azerbaijan. 33 rare species included in the first edition were removed from the second edition for various reasons. The second edition of the "Red Book" describes 330 species, including 266 higher (1 species of mosses) and 20 primitive plants, 14 species of fungi.

The first edition of the "Red Book" includes 108 animal names, of which 14 are mammals, 36 are birds, 13 are reptiles and amphibians, 5 are fish, and 40 are insects. The second edition of the "Red Book" includes 223 species of animals that are rare, endangered, and in need of protection, of which 72 belong to the class of birds and 77 to the class of insects.

It is known that according to Decree No. 186 of the President of the Republic of Azerbaijan dated August 30, 1999, on the implementation of the Law of the Republic of Azerbaijan "On the Animal World", and following the Regulation adopted by the Decision No. 125 of the Cabinet of Ministers of the Republic of Azerbaijan dated July 15, 2000, the publications of the "Red Book" are organized by the Ministry of Ecology and Natural Resources of the Republic of Azerbaijan in connection with the National Academy of Sciences of Azerbaijan. According to this regulation, the "Red Book" of the Republic of Azerbaijan must be revised and published no later than 10 years.

To remove from the list, the species that were included in the I and II editions of the "Red Book" and due to the protection measures carried out during the past period, a significant increase in the number and range of which was recorded, as well as species that had not been found in the flora and fauna of the country for a long time, while their names were on the list, besides, taking into account the serious need to add species whose numbers and distribution were significantly reduced, activities on the new – the III edition of the "Red Book" were intensified in 2019.

In this regard, to resolve the issues faced by the Azerbaijan National Academy of Sciences, the relevant decisions of the ANAS Presidium dated October 16, 2019, and January 9, 2020, were adopted, and the ANAS editorial board of the publication was formed. The editorial staff includes well-known scientists, specialists, and leaders in relevant fields.

The main issues for the III edition of the "Red Book" were planned as follows: definition of preliminary lists; collection of field materials (also herbarium for plants); clarifying the taxonomic status of rare and endangered species by evaluating them according to international and local categories and criteria; creating an electronic database; taking pictures of species and preparing distribution maps; essay writing, book design.

Taking into account the results of the discussions organized and coordinated by the Division of Biological and Medical Sciences of ANAS, the Editorial Board of "Red Book" prepared a work program covering the years 2019-2023 for the 3rd edition and approved by decision No. 8/9 of the Presidium of ANAS dated February 14, 2020. Local and international experience, the experience of the CIS countries and Turkey were taken into account in the preparation of the program.

All possible activities in the implementation of the work program were carried out by the relevant editorial staff of ANAS, working groups made up of scientists and specialists of the institutes of Botany, Zoology, Dendrology, Nakhchivan Bioresources, the Central Botanical Garden, and other scientific research and educational institutions. The preparation, verification, and clarification of the status of the red lists of plants, fungi, and animals were carried

out within the framework of both thematic plans of the institutes and national strategies and action plans adopted several times over the past 20 years for the protection and sustainable use of biological diversity in the republic.

Following the “Regulations on the "Red Book" of the Republic of Azerbaijan”, on October 16, 2020, an agreement was signed between the Azerbaijan National Academy of Sciences and the Ministry of Ecology and Natural Resources of the Republic of Azerbaijan on cooperation and division of labor regarding the third edition. In 2019-2021, the editorial staff and working groups of ANAS operated under the strict supervision of the Division of Biological and Medical Sciences of ANAS.

The work of the groups was led by the director general of the Institute of Botany, MSE AR, doctor of biological sciences, Professor Sayyara Ibadullayeva and the department head of the institute, doctor of biological sciences Aydin Asgarov, acting director of the Institute of Zoology, MSE AR, doctor of philosophy in agricultural sciences, Associate Professor Aladdin Eyvazov, executive director of the same institute, doctor of biological sciences, Associate Professor Elshad Ahmadov.

Regularly meetings were held at scientific research institutions of DBMS and Bioresources Institute of Nakhchivan Department of ANAS (currently these institutes are included in the structure of the Ministry of Science and Education of the Republic of Azerbaijan), Baku State University, Ganja State University, the representative office of the World Wildlife Fund for Nature (WWF) in Azerbaijan, in the Azerbaijan Ornithological Society with the involvement of botanists and zoologists working in other scientific and educational institutions, extensive discussions were held, existing plans were clarified, and decisions were made. Most of the meetings were attended by employees of the Ministry of Ecology and Natural Resources of the Republic of Azerbaijan, and in several meetings, the Minister of Ecology and Natural Resources Mukhtar Babayev, and other responsible persons participated.

Relevant seminars and training courses were also held during the past period, considering the need for specialists to become familiar with international experience and acquire deeper knowledge regarding the evaluation of the species planned to be included in the III edition of the "Red Book" of the Republic of Azerbaijan according to the categories and criteria of the IUCN Red List. In this regard, the training course organized jointly by the Azerbaijani representative office of the WWF and the Institute of Zoology, on October 17-19, 2022, should be especially mentioned. Commission member of IUCN, ecologist Dr. Andrew Rodrigues was invited to the course, where members of the working groups of various organizations (Ministry of Ecology and Natural Resources of the AR, Institutes of Botany and Zoology, etc.) involved in the preparation of the III edition of the "Red Book" participated.

The three-day training covered topics, including the process of determining taxonomic ranks and categories, diversity and uniqueness, the nature of categories, the role of various criteria, protective measures in the process of compiling the Red List, completeness of data, the importance of scientific results and predictions, ecological importance and interactions between species, rules for using imprecise data for taxon assessment, scaling issues, taxon assessment at the global, regional or national level, taxon mapping across geographical areas, etc.

On November 22, 2022, the relevant decision of the ANAS Presidium regarding the new structure of the Editorial Board of the III edition of the "Red Book" was adopted. Academician Isa Habibbayli, the president of ANAS, was appointed the chairman of the editorial board by ANAS. The person responsible for the scientific development of the book and the editors responsible for flora and fauna were also mentioned in the decision.

Activities on the third edition of the "Red Book" of the Republic of Azerbaijan, large-scale scientific research was carried out on the basis of a program developed by botanic and zoological scientists and specialists of Azerbaijan, taking into account local and international experience and involving modern research methods.

First, the meetings of the Editorial Board of the "Red Book" and the working groups created in the Institutes of Botany and Zoology of the MSE AR were held, taking into account the studies and literature data of the last years, as well as the previous editions of the book, rare and endangered

plants were selected for inclusion in the III edition, approximate lists of fungi and animal species have been prepared again. At the meetings, proposals for delisting species that have lost conservation importance and inclusion of endangered species were discussed. The general decisions were made based on the broad geography, taxonomy, morphology, and other characteristics of each species, as well as the global general knowledge of experts on that species.

Then, with the help of scientific monitoring carried out in all regions of the republic along the research routes, the current status of rare and endangered plant, fungal, and animal species, their population structure, composition, and number of individuals, and their distribution were clarified, and areal maps were drawn up.

Based on the compiled lists, information about the populations of species in their distribution areas, including GPS coordinates, was collected based on common descriptors. In order to determine the factors of external influence, the monitoring was carried out according to the bioecology of the respective species and the seasons. Unlike the previous two editions of the "Red Book", this time, on the basis of the collected data, species were evaluated according to the categories and criteria of the International Union for Conservation of Nature (IUCN).

In multidisciplinary studies, modern information and computer technologies, aerospace data, mathematical and statistical modeling methods were widely used in predicting changes in priority plant, fungal, and animal species and developing future scenarios.

A large volume of scientific and technical information collected during the 4-year research period, some of which fell into the period of the COVID-19 pandemic and quarantine, was analyzed and processed, the relevant materials were translated into English and the third edition of the "Red Book" was compiled, and text, maps, and images were edited.

In the book's layout, each target species is described on one page. This page reflects the results of the international and national assessment according to the IUCN categories and criteria, the description of the species, bioecological characteristics, distribution, limiting factors, existing and proposed conservation measures (the last 4 indicators are also given in English). A photo of the species on the page and a distribution map provide visibility. Information about the description of the species, bioecological characteristics, and distribution are confirmed with references to relevant literature sources.

Limiting factors for most species include mainly anthropogenic (overharvesting, hunting and grazing, expansion of anthropogenic landscapes, fragmentation, use of herbicides, etc.), and partly natural and semi-natural factors (climate changes, mass impact of pests and invasive species, etc.).

It is known that the territories occupied by Armenia for up to 30 years were not sufficiently reflected in the second edition of the "Red Book" of the Republic of Azerbaijan. Because it was impossible to carry out any research, monitor, and determine the real situation of rare and endangered species and their populations in the region. Rare pearls of nature included in the "Red Book" of the Republic of Azerbaijan and the "Red List" of the International Union for Conservation of Nature were destroyed, medicinal plants were collected, and valuable dendroflora samples (Oriental sycamores (*Platanus orientalis* L.), red oak (*Quercus rubra* L.) included in the "Red Book" were cut and transported away. The fate of the flora and fauna diversity of the specially protected natural areas with an area of 43 thousand hectares - Basitchay and Garagol state nature sanctuaries, Arazboyu, Lachin, Gubadli, and Dashalti state nature reserves included in the "Red Book" of the Republic of Azerbaijan was questionable.

After the liberation of our lands under the leadership of Commander-in-Chief, President Ilham Aliyev, and thanks to the bravery of the Azerbaijani soldier, it was possible to involve the region in research on the III edition of the “Red Book”.

One of the main topics of the international conferences organized by the Division of Biological and Medical Sciences of ANAS in 2021-2022 and held with great success ("Biodiversity, land, and water resources of Karabakh: past, present and future", May 20-21, 2021; Karabakh- II International congress of applied sciences dedicated to Victory Day and the dear memory of our martyrs, November 8-10, 2021; " “Biodiversity, soil and water resources of

Shusha and adjacent territories: a look into the future", September 22-23, 2022), was related to the red lists of the Karabakh region, rare and endangered species, and the problems of their protection.

Fifteen species of the fauna of Karabakh, always distinguished by its richness, had been included in the second edition of the "Red Book" of the Republic of Azerbaijan. In the territories occupied by Armenia for nearly 30 years, 56 species of insect fauna are rare, endemic, and in danger of extinction. It should be noted that most of them are beneficial insects.

There are 12 species of fish that were once widely used in the diet of the local population in the internal water basins of Karabakh, 7 of which are included in the Red List of the IUCN along with the II edition of the "Red Book".

The fauna of birds in the Lesser Caucasus was particularly rich. Until 1993, 288 species of birds belonging to 16 orders and 57 families were registered in the territory of Karabakh (for comparison, there are 407 species of birds belonging to 19 orders and 65 families in the territory of Azerbaijan). Of these, 50 species were included in the second edition of the "Red Book".

Before the occupation, 64 species of mammals belonging to 6 orders (115 in Azerbaijan) were recorded in the territory of Karabakh. Some of them are included in the Red List of the International Union for Conservation of Nature, as well as in all editions of the "Red Book" of the Republic of Azerbaijan.

Many of the 266 rare and endangered species of higher plants described in the first two editions of the "Red Book" of the Republic of Azerbaijan, including a number of species endemic to the region, such as khari bul bul, platanus, and oak are found in Shusha and the surrounding areas.

Our scientists worked selflessly in the expeditions conducted for the exploration of the flora and fauna samples included in the "Red Book" in the territories freed from occupation.

Sayyara Ibadullayeva, director general of the Institute of Botany, MSE AR employees of the institute, PhD in biology Nuri Movsumova, PhD in physics and mathematics Elman Yusifov, head of the laboratory of the Institute of Zoology of MSE AR, PhD in biology Tavakkul Iskanderov, researcher Elshad Asgarov, head of the laboratory of the Institute of Microbiology, doctor of biological sciences, professor Elman Isgandar, other scientists and specialists conducted research in separate areas of the region cleared of mines.

Red kite (*Milvus milvus* L., 1758), Mehely's horseshoe bat (*Rhinolopus meheli* Matschie, 1901), European free-tailed bat (*Tadarida teniotis* Raf., 1814; In Azerbaijan, it was recorded only in the Shusha valley), Olivier's agama (*Trapelus ruderatus* Olivier, 1804), Eastern rock-nuthatch (*Sitta tephronota* Sharpe, 1872), Karabakh tulip (mountain tulip) (*Tulipa karabachensis* Grossh.), Karabakh psephellus (*Psephellus karabaghensis* Sosn. (=*Centaurea karabaghensis* (Sosn.) Sosn.); recorded only around the city of Khankendi), Caucasian zelkova (*Zelkova carpinifolia* (Pall.) Dippel), Alcea (*Alcea sachsachanica* Iljin), Pulsatilla (*Pulsatilla violacea* Rupr.), Shusha astragalus (*Astragalus schuschaensis* Grossh.), Steppe peony (*Paeonia tenuifolia* L.; found only in Zangilan, Basitchay State Nature Reserve), Orange day-lily (*Hemerocallis fulva* (L.) L.), and dozens of other animal and plant species related to fauna and flora of the Karabakh territory have been included in the third edition of the "Red Book".

Decisions made and implemented, numerous measures, purposeful work, conducted expeditions and camera studies, high attention and control of the management over the entire process gave positive results, and the III edition of the "Red Book" was elegantly published.

The current edition of the "Red Book" includes 241 rare and endangered species of fauna (152 vertebrates, 89 invertebrates; 39 mammals, 78 birds, 18 reptiles, 11 fish, 7 aquatic invertebrates, 82 insects, 6 amphibians) and 460 species of flora (423 plants: 383 higher plants, 15 higher with spores, 6 mosses, 14 lichens, 5 algae; and 37 fungi).

CONCLUSION

In conclusion, it is important to note that a large creative team worked on the third edition of the "Red Book" of the Republic of Azerbaijan. The book was created thanks to the great and hard work of the editorial staff, working groups, the institutes of Botany, Zoology and Dendrology, Ministry of Science and Education of the Republic of

Azerbaijan (MSE AR), Baku State University, Nakhchivan Institute of Bioresources of the MSE AR, Ganja State University, Azerbaijan Society of Ornithologists, Central Botanical Garden, etc., researchers of organizations in relevant fields, dozens of compilers, experts in information technologies, design, mapping, translation, editing, etc. The publication is dedicated to the "Year of Heydar Aliyev" marked by the Decree of the President of the Republic of Azerbaijan dated September 29, 2022, and is a valuable contribution of the creative team to the glorious jubilee.

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